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Thermal processing techniques for improving protein-enriched beverage production

Thesis presented to the National University of Ireland
for the degree of Doctor of Philosophy by

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Declaration by the Candidate

Thermal processing techniques for improving

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This is to certify that the work I am submitting is my own and has not been submitted for another degree, either at University College Cork or elsewhere. All external references and sources are clearly acknowledged and identified within the contents. I have read and understood the regulations of University College Cork concerning plagiarism.

Clodagh Kelleher

16th January 2019

Dedication

To those who inspired it and will never read it

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Publications

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Kelleher, C.M., O'Mahony, J.A., Kelly, A.L., and O'Callaghan, D.J., Viscosity-temperature profiles of milk protein beverage models. *44th Annual Food Conference*, Teagasc Moorepark, Cork, 14th December 2015.

Kelleher, C.M., O'Mahony, J.A., Kelly, A.L., and O'Callaghan, D.J., Modelling the temperature-dependence of the viscosity of model dairy protein beverage formulations of differing protein and carbohydrate levels. *IDF Dairy Science and Technology Symposium,* Dublin, 11 – 13th April 2016.

Abstract

Growing consumer demand for convenient sources of high quality protein has led to a substantial global market for ready-to-drink, shelf-stable dairy protein beverages. These products have significant technical challenges associated with their manufacture due to the high processing temperatures required to render them microbiologically stable. Issues arise during thermal processing and storage as the high protein content leaves formulations susceptible to protein denaturation and aggregation, development of volatile compounds with associated off-flavours, increased viscosity, and sedimentation. This has driven dairy processors to seek out alternative formulation approaches and thermal processing techniques which can minimise the thermally-induced changes in the product, while still achieving the required shelf life. Relatively few studies have focused on a combined approach of investigating varied protein profiles in protein-enriched beverages with a range of thermal processing techniques.

The objective of this research was to investigate the impact of composition and processing parameters on the physical behaviour of high-protein milk beverage systems across a range of thermal treatment systems. A new methodology for assessing the thermal stability, in terms of viscosity, for protein beverage formulations was also developed. Protein profile was shown to affect thermally-induced protein denaturation, with reductions in α-lactalbumin denaturation with an increasing casein proportion. Alterations in preheat treatment temperature significantly affected viscosity in protein systems upon concentration. The application of temperature-dependent viscosity models was demonstrated to be a useful, rapid tool in quantifying differences in product processing stability. Direct heating technology was applied to ESL- and UHT-treated whey protein solutions with a high protein content (4, 6 and

8% protein (w/w)) resulting in reduced protein denaturation, viscosity and less extensive changes to the volatile profile compared to tubular heating. A pilot-scale supersonic steam injection line was designed and integrated into an existing tubular heating plant with commissioning completed on skim milk. This novel heating system provided significantly greater reductions in protein denaturation than direct steam infusion and tubular heating for protein-enriched dairy beverage systems.

The thesis provides new insights into interactions between milk proteins during thermal processing which influence the physical and volatile profile stability of protein beverages. The outcomes of the work have applications in such areas as high heat treatment processing of heat-sensitive milk proteins, allowing for minimised whey protein denaturation, reduced viscosity through formulation manipulation, and use of novel thermal technologies.

Nomenclature

Abbreviation	*Description*
a_T	Ratio between viscosity at temperature, T and viscosity at selected temperature, T_s
α	Alpha
α-la	α-lactalbumin
β	Beta
β-lg	β-lactoglobulin
γ	Gamma
$\dot{\gamma}$	Shear rate
ΔE	Euclidean colour difference
ΔH	Enthalpy of denaturation
κ	Kappa
μ	Viscosity
μL	Microlitre
μm	Micrometer
μ_r	Relative viscosity
μ_s	Volume of continuous phase
μ_T	Apparent viscosity at temperature T
σ	Shear stress
ϕ	Volume fraction
$\frac{dv}{dx}$	Velocity gradient
~	Approximately
°C	Degrees Celsius

1/s	Reciprocal seconds
A	Area
ANOVA	Analysis of variance
BCAA	Branched chain amino acids
BSA	Bovine serum albumin
C_1, C_2	Williams Landel Ferry constant
Ca^{2+}	Calcium ion
CCP	Colloidal calcium phosphate
CFD	Computational fluid dynamics
CIP	Cleaning-in-place
cm	Centimeter
CN:WP	Casein to whey protein ratio
Da	Dalton
DLS	Dynamic light scattering
DLS	Dynamic light scattering
DSC	Differential scanning calorimetry
E_a	Activation energy
ESL	Extended shelf life
F	Force
FF	Fluid food
g	grams
GC-MS	Gas chromatography mass spectrometry
GHz	Gigahertz
GMP	Glycomacropeptide
HCl	Hydrochloric acid

HCl	Hydrochloric acid
HF	Heated food
HHP	High hydrostatic pressure
HPH	High pressure homogenisation
HPLC	High performance liquid chromatography
HPP	High pressure processing
HPTP	High pressure thermal processing
hr	Hours
HS-SPME	Head space solid phase microextraction
HTST	High-temperature short-time
Hz	Hertz
Ig	Immunoglobulin
J	Joule
K	Consistency coefficient
K	Kelvin
kDa	Kilo Dalton
Kg	Kilogram
kJ	Kilojoule
KOH	Potassium hydroxide
KOH	Potassium hydroxide
kV	Kilovolt
L	Litre
LSI	Lenient steam injection
LTLT	Low-temperature long-time
M	Molar

m	Meter
Ma	Mach number
MHz	Megahertz
min	Minutes
mL	millilitre
mm	Millimetre
mM	Millimolar
mol	Mole
mPa	Millipascal
MPa	Megapascal
MPC	Milk protein concentrate
MPI	Milk protein isolate
MW	Molecular weight
MWH	Microwave heating
n	Behaviour flow index
N	Newton
NaCl	Sodium chloride
NaOH	Sodium hydroxide
nm	Nanometer
OH	Ohmic heating
Pa	Pascal
PCA	Principal component analysis
PDCAAS	Protein-digestibility corrected amino acid score
PEF	Pulsed electric field
PHE	Plate heat exchanger

pI	Isoelectric point
PP	Proteose-peptone
PPC	Post-processing contamination
ppm	Parts per million
R	Universal gas constant
rad	Radians
Re	Reynolds number
RP-HPLC	Reverse phase high performance liquid chromatography
rpm	Rotations per minute
S	Steam
s	Seconds
SE-HPLC	Size exclusion high performance liquid chromatography
-SH	Free sulfhydryl group
SMP	Skim milk powder
SSHE	Scraped surface heat exchanger
SSR	Sum of squared residuals
T	Temperature
T_D	Initial denaturation temperature
T_g	Glass transition temperature
THE	Tubular heat exchanger
T_s	Selected temperature
T_{tr}	Temperature at the differential scanning calorimetry peak maximum
UHP	Ultra-high pressure
UHT	Ultra-high temperature

UP	Ultra-pasteurisation
w/v	Weight by volume
w/w	Weight by weight
WLF	Williams Landel Ferry
WPC	Whey protein concentrate
WPI	Whey protein isolate

Chapter 1.

Literature Review: Thermal processing technologies and their application to protein-enriched beverages

1.1. Introduction

Protein-enriched dairy beverages are important to the Irish economy and the worldwide market as a significant part of the lucrative nutritional and performance beverage market. Increasing health awareness and consumer demands for clean label, minimally processed, fresh tasting, nutritionally-fortified foods which are convenient, cost-effective and have long shelf lives are posing substantial technical challenges for food producers (Huang *et al.*, 2017). As a result there has been a growing interest in formulation development and preservation techniques which can minimise thermally-induced changes to the physical characteristics, nutritional quality and sensory profile of dairy systems during manufacture.

There has been little research into the impact of different thermal processing techniques on final product quality of protein-enriched beverages, with many studies focusing on the application of laboratory-scale heating methodologies (LaClair and Etzel, 2010; Chen and O'Mahony, 2016; Wagoner and Foegeding, 2017; Chevallier *et al.*, 2018). This review provides a context and background to these nutritional products, with a focus on beverage formulation and associated market trends, thermal processing requirements, and typical thermally-induced changes which can pose technical challenges in their manufacture. The different forms of thermal and non-thermal preservation technologies are also reviewed in order to understand differences in their modes of operation and the outcome of their application to different dairy products.

1.2. Dairy Proteins

Bovine milk and its derived dairy products have long-standing traditions and prevalence in human nutrition, with historical references to milk dating back as far as

4000 BC (Jensen and Kroger, 2000; Miller *et al.*, 2007). Today, dairy products are consumed by over 50% of the world's population daily and world milk production is estimated at over 600 million tons, with 85% of this being produced from cows (Haimov-Kochman *et al.*, 2016; Fox, 2001).

The major components of bovine milk are water, fat, lactose, protein and minerals. Concentrations of these components may vary due to a number of factors such as breed, health, nutritional status, stage of lactation, age, interval between milking etc. (Ontsouka *et al.*, 2003; Fox *et al.*, 2017). The protein content, approximately 3.4 g/100 mL, is divided into two fractions; casein and whey protein. In mature milk, caseins represent approximately 80% of the total protein content, while the remaining 20% consists of whey protein. These fractions are easily separated at the isoelectric point (pI) of caseins at pH 4.6 at 20 °C, at which pH caseins coagulate and precipitate while whey proteins remain soluble (Huppertz, 2013; Fox *et al.*, 2017a), or on a molecular weight basis using membrane filtration technology (Kumar *et al.*, 2013).

The casein fraction is subdivided into αs_1-, αs_2-, β-, and κ- casein in the approximate ratio of 38:10:35:15 (Holt and Sawyer, 1993; Fox and McSweeney, 1998). These caseins are a heterogeneous group of phosphoproteins which self-assemble into colloidal protein aggregates known as casein micelles (Juszczak and Fortuna, 2006). The phosphate groups of these phosphoproteins are esterified to serine residues, which are important to the casein micelle structure (Fox, 2001; Roos, 2002; Lewis and Deeth, 2009). While caseins are small proteins with a molecular weight of 20 - 25 kDa, casein micelles are much larger with a size range of 30 - 300 nm (10^8 - 10^9 Da). Due to their light-scattering properties, these coarse colloidal particles are responsible for the turbid white appearance of milk (Walstra *et al.*, 1999; O'Mahony and Fox, 2013).

On a dry weight basis, the micelles consist of ~94 % protein and ~6 % low molecular weight salts, primarily calcium, phosphate, magnesium and citrate, often referred to collectively as colloidal calcium phosphate (CCP) (Roos, 2002). CCP is important for neonate nutrition and one of the main functions of the casein micelle is to act as a sequestrant of insoluble CCP (Holt *et al.*, 1996; Holt *et al.*, 1998).

Due to the high level of proline present in all caseins, particularly β-casein, they have little secondary structure (α-helices and β-sheets) and instead have a flexible, mobile conformation, i.e. rheomorphic, structure (Fox, 2001). This lack of secondary and tertiary structure gives caseins good emulsifying and foaming properties, and high heat stability (140 °C for 20-25 min), however, caseins are very susceptible to proteolysis (Fox, 2001; Smithers, 2008). Proteolysis of β-casein by the indigenous protease plasmin can result in γ_1-, γ_2-, and γ_3-casein and proteose-peptones (PP), while proteolysis of κ-casein by chymosin can produce para-κ-casein and glycomacropeptide (GMP) (Kelly and McSweeney, 2003; Andrews, 1978; Mulvihill and O'Donovan, 1987; Swaisgood, 1993).

The precise structure of the casein micelle is much debated, with numerous models being proposed over the years (Fig. 1.1). However, all models agree that most of the κ-casein, representing ~12 – 15 % of total casein, is present on the surface of the micelle. The hydrophilic, C-terminal of κ-casein is thought to protrude 5 - 10 nm from the surface of the micelle into the surrounding solvent, preventing the approach and interactions of hydrophobic regions of the casein molecules (Roos, 2002). This placement of κ-casein allows for stabilisation of calcium-sensitive αs_1-, αs_2- and β-caseins through electrostatic and steric repulsion, which would otherwise precipitate in the 30 mM Ca^{2+} environment of milk (Crowley *et al.*, 2016).

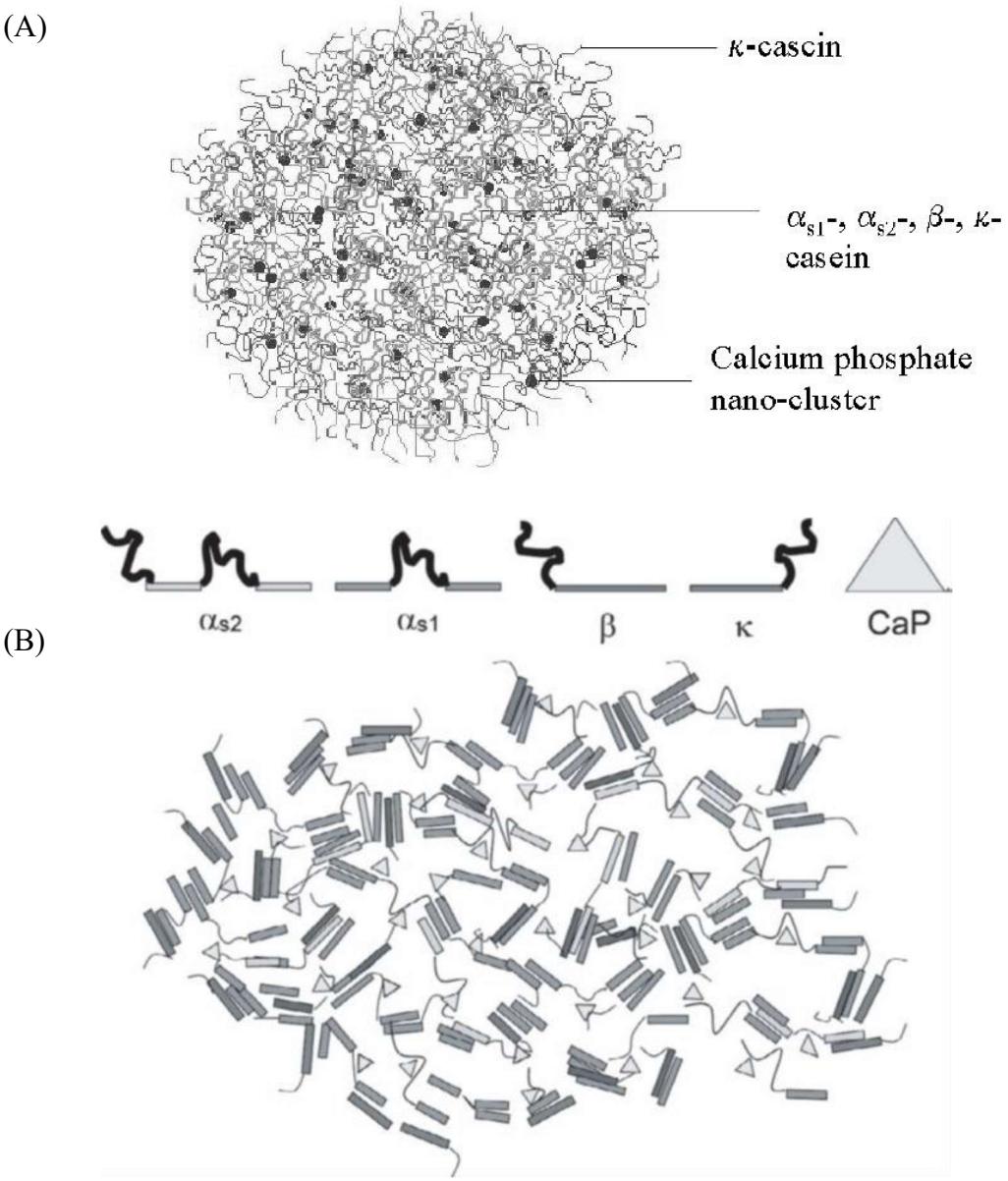

Fig. 1.1. (A) Hairy casein micelle model proposed by Holt (Holt, 1992) and (B) the dual binding model with hydrophobic regions presented as rectangular bars proposed by Horne (Horne, 1998).

Whey proteins are globular proteins with high levels of secondary, tertiary and even, quaternary structure, making them sensitive to denaturation upon heating at temperatures exceeding 70 °C (Fitzsimons *et al.*, 2007). Unlike casein, these proteins are not phosphorylated and are insensitive to Ca^{2+} (Fox, 2001; Elliot *et al.*, 2003). The principal fractions of whey proteins are β-lactoglobulin (β-lg, 50 %), α-lactalbumin

(α-La, 20 %), bovine serum albumin (BSA, 10%), and immunoglobulins (Ig, 10 %; mainly IgG_1) (Fox, 2001; Roos, 2002).

α-La is a small protein with a molecular weight of 14.2 kDa that forms part of the lactose synthase enzyme, involving galactosyl transferase with glucose as the preferred substrate, and in the regulation of lactose biosynthesis (Mulvihill and Donovan, 1987). The amount of lactose in milk has been found to be directly correlated with the quantity of α-la (Brodkorb *et al.*, 2016). This protein is stabilised by disulphide bonds which allows for reversible denaturation, in which the protein can refold close to its native form after unfolding due to thermal denaturation. However, instability can result in the presence of other proteins, such as β-lg, where cross links between the unfolded proteins can be formed, resulting in permanent denaturation and aggregation (Wallhäußer *et al.*, 2012).

β-Lg is the most prominent of the whey proteins, accounting for ~50 % of the total whey protein in bovine milk (Roos, 2002). It is a globular protein with a molecular weight of 18.3 kDa (O'Kennedy and Mounsey, 2009), and it is composed of 162 amino acid residues, including a relatively high proportion of branched-chain amino acids (BCAAs). β-Lg contains 22 leucine, 10 isoleucine, and 9 valine residues in the molecule, making it one of the richest sources of these amino acids and providing strongly hydrophobic regions of the protein.

β-Lg is susceptible to heat-induced denaturation and aggregation. Factors influencing this include non-covalent interactions such as ionic, van der Waals and hydrophobic interactions. The reversibility of this aggregation is dependent on the degree of chemical and physical aggregation, in addition to factors such as temperature, pH and

ionic strength (O'Kennedy and Mounsey, 2009). β-Lg is present in two genetic variants, A and B which can be separated by ion chromatography (Sawyer, 2003).

The amount of BSA present in milk and in whey can vary. BSA is passively leaked into the milk from the blood serum at tight junctions in the cells of the mammary gland. The amount of serum albumin leaked into the milk is dependent on the health of the cow and particularly the mammary gland; however, it is generally present at low levels (~10 %; O'Mahony and Fox, 2013).

The Igs found in bovine milk are a mixture between those synthesised in the blood and in the mammary gland. Igs are a complex mixture of glycoproteins which possess antibody activity. These proteins are found in high concentrations in colostrum (6 % w/v) but are present in significantly lower levels (0.06 - 0.10 % w/v) in milk obtained from later lactation stage milk (Mulvihill and Donovan, 1987. There are four classes of immunoglobulins; IgM, IgA, IgE and IgG, which is present as both IgG_1 and IgG_2. The general structure of immunoglobulins is either a monomer or polymer of a four-chain molecule consisting of two light and two heavy polypeptide chains (Mulvihill and Donovan, 1987). They are typically globular proteins and are heat-labile, although this generally does not greatly affect the product in such low concentrations.

1.3. Nutritional quality of dairy protein

Milk is a complex liquid food system, designed to meet the full nutritional requirements of the neonate, and as a result, it is one of the most complete single foods available in terms of nutrition (O'Mahony and Fox, 2013; Fox *et al.*, 2017a). A cost-effective source of high quality nutrition, milk has the lowest overall nutrient-to-price ratio, lowest-cost source of calcium and is among the lowest-cost sources of protein (Pellegrino, 2013). Milk is a good source of essential amino acids, unsaturated fatty

acids, vitamins, minerals, and bioactive peptides (Claeys *et al.*, 2013). The essential amino acids provided include all nine that the human bodies cannot synthesize, which support muscle recovery and repair (Miller *et al.*, 2007; Wijayanti *et al.*, 2014; Chen and O'Mahony, 2016). Proteins in milk are considered one of the best protein types in terms of digestibility and bioavailability, with high protein-digestibility corrected amino acid score (PDCAAS) (Huag *et al.*, 2007; Pereira, 2014). Due to its high nutritional value and the ease with which it can be processed into a wide range of products, milk and its derivatives feature extensively in the human diet, supplying around 30% of dietary protein in industrialised counties such as the USA, Canada, New Zealand, Australia and many European countries (O'Mahony and Fox, 2013).

Caseins are a good source of high-quality protein, with high proportions of histidine, methionine and phenylalanine. They are also regarded for a high mineral-binding capacity, acting as a carrier for calcium and phosphorous in the form of CCP (Pereira, 2014). Milk is an important source of dietary calcium, associated with improving bone density and reducing the risk of osteoporosis (Wood, 2003). Calcium has also been linked to protection against hypertension and cancer, while phosphorous plays an important role in metabolism (Miller *et al.*, 2007). Bioactive peptides are produced through the proteolysis of casein molecules; these are short amino acid chains which exhibit antimicrobial activity (Ebringer, Ferencik, and Krajcovic, 2008; Claeys *et al.*, 2013; Pellegrino *et al.*, 2013).

Whey proteins have a very high biological value, 15 % greater than that of egg protein, where the biological value is a measure of the percentage of protein utilised by the body (Smithers, 2008). They are rich in branched-chain amino acids (BCAA, ~26%), such as leucine, isoleucine and valine, which support tissue growth and repair (Ha and Zemel, 2003; Beucler, 2005; Smithers, 2008; Gupta, 2012). Leucine plays a role in

insulin and glucose metabolism (Gupta, 2012), and valine has been linked to weight control as a metabolic regulator in protein and glucose homeostasis, and lipid metabolism (Smithers, 2008). Studies have shown that BCAAs can provide energy during prolonged exercise periods and prevent loss of body mass and muscle (Gupta and Prakash, 2015). Studies have shown that whey proteins may have health-promoting properties such as promoting beneficial gut microflora, immune-enhancing properties, improved glycaemic control reducing risk of cancer, and anti-toxin activity (LaClair and Etzel, 2010; Gupta and Prakash, 2015). The high content of sulphur-containing amino acids in whey proteins support anti-oxidant functions (De Wit, 1998, Sinha *et al.*, 2007; Gupta and Prakash, 2015). Whey proteins have been used in the treatment illnesses like sarcopenia, loss of lean body mass associated with aging, as they can positively impact muscle synthesis rates (Gupta and Prakash, 2015). Milk- and whey-derived peptides have also been linked to increased satiety and weight loss (Ha and Zemel, 2003). Whey proteins can also promote levels of glutathione (GSH), an anti-oxidant required for a healthy immune system and successful exercise (Gupta, 2012). Carbohydrate-based sports beverages containing whey protein have been shown to reduce muscle soreness in athletes compared to carbohydrate-only beverages (Millard-Stafford *et al.*, 2005).

In the past, whey was considered a waste by-product of cheese and casein production, with companies choosing to dispose of or sell as animal feed for a low return (Smithers, 2008). Over the past 30 years, whey proteins have become an increasingly important ingredient due to their nutritional, functional and in some cases pharmaceutical properties, instead becoming a valuable co-product (Mulvihill and Donovan, 1987; Wallhäußer *et al.*, 2012; Fox *et al.*, 2017b). Increased consumer awareness and changing requirements have led to market demands for healthy, tasty

and convenient foods and the creation of new opportunities for whey-based ingredients, enabled by technological developments for the economic recovery of whey protein (Mulvihill and Donovan, 1987; Smithers, 2008). These are used in a wide range of products including sports beverages, infant nutrition formulae, liquid meat replacements, baked products, processed meats, salads dressings, ice creams, artificial coffee creams, soups and various dairy products (Dissanayake, 2009).

Casein and whey proteins have different PDCAAS and biological values as their mechanisms of digestion differ. Boirie *et al.* (1997) described differences in the digestion and absorption of casein and whey proteins, considering them to be 'slow' and 'fast' proteins, respectively. This difference is due to the coagulation of casein in the stomach, due to precipitation by gastric acid, resulting in a longer overall gastric emptying time and a reduced increase in plasma amino acid release compared to whey protein (Hall *et al.*, 2003; Lacroix *et al.*, 2006). Due to the higher post-prandial levels of amino acids released, the 'fast' whey proteins have been shown to be more satiating than the 'slow' caseins. These digestion mechanisms and satiety capacities can be considered when incorporating dairy proteins into products, with fast, satiating proteins being used for rapid nutrition in high performance sports or for weight management treatments, while slow proteins could be used for clinical nutrition products with the aim of increasing food intake (Hall *et al.*, 2003; Oltman *et al.*, 2015). However, studies have found that due to the 'fast' release of whey protein can result in incomplete digestion of the available amino acids (Lacroix *et al.*, 2006; Sah *et al.*, 2016). The digestion of dairy proteins can be modulated and the release of BCAA's prolonged by co-ingesting protein with carbohydrates, utilising an emulsion matrix, or thermal treatment (Sah *et al.*, 2016).

Thermal treatment can result in whey proteins being involved in the coagulation of casein in the stomach, altering the rate of digestion as casein digestion is increased while whey protein digestion is slowed (Ye *et al.*, 2015). However, technological processes used to manufacture milk products may impair this high nutritional value, where protein denaturation, subsequent aggregation and loss of solubility decreases protein digestibility and the availability of substrate to enzymatic digestion (Lacroix *et al.*, 2006; Ye *et al.*, 2015; Claeys *et al.*, 2013; Pellegrino, 2013). Preserving the bioactivity of these milk components may require new processing technologies that can provide preservation while limiting thermal denaturation (Tong and Smithers, 2013).

Increasingly health-conscious consumers are demanding high quality, minimally processed, clean label foods which have a high nutritional value, fresh flavour and are free from chemical additives. This poses significant technical challenges for food producers and increases interest in formulation development and preservation techniques which limit negative effects on the physical characteristics, nutritional quality and sensory attributes of food during manufacture (Huang *et al.*, 2017).

1.4. Dairy protein-enriched beverages and current market trends

Consumers have an increased interest in healthy food and beverages with functional properties that can provide them with added nutritional benefits (Ozen *et al.*, 2012; Corbo *et al.*, 2014; Chevallier *et al.*, 2018). The US nutrition and performance drinks market was worth $14 billion in 2017, a 26 % increase from 2012 (Mintel, 2018). The positive effects of dairy proteins are utilised in protein-fortified dairy beverages to develop sports performance drinks which impact positively on exercise-induced muscle damage (Shiby, 2013; Chen and O'Mahony, 2016). Studies have shown that

the administration of as little as 6 g of essential amino acids after a strength training exercise session can increase the rate of protein synthesis (Maughan, 2009). In order to meet FDA standards for "high" or "excellent source of" protein label claims, beverages are required to contain at least 10 g of protein per serving, or 4 % protein per 250 mL (Wagoner and Foegeding, 2017). An average protein content of 8.6 % (w/w) was determined from 250 commercial dairy protein-enriched beverages across 49 countries (Mintel, 2018). A review by the author of 55 commercially available ready-to-drink, shelf-stable milk protein beverages available in Ireland, revealed that the average protein content was lower than the global value at 6.81 %. The most common protein ingredients used in the manufacture of these products were milk protein concentrate, whey protein isolate and whey protein concentrate.

Dairy protein beverages are generally divided into two types, acidic (≤ pH 4.6) and low acid/neutral pH (≥ pH 4.6), each with their own technical difficulties in terms of production. For acidic beverages, turbidity and astringency after thermal processing is a concern, while, for neutral pH beverages, off-flavours and thermal instability are the main issues (LaClair and Etzel, 2010; Wagoner and Foegeding, 2017). Acidic beverages require a lower level of thermal processing to ensure safety and shelf stability and are processed at temperatures below 100 °C, while low acid/neutral beverage require thermal processing above 100 °C (Ryan and Foegeding, 2015). Due to the low pH of acidic beverages, there is a low risk of microbial growth, allowing for reduced thermal processing conditions. The flavours of protein-enriched beverages are also guided by the pH of the systems; neutral pH beverages are generally opaque with creamy vanilla and chocolate flavours, while acidic beverages are clear and commonly feature more fruity flavours such as grape, lemon-lime and fruit punch

(Beecher *et al.*, 2008). Neutral pH protein beverage flavours are more prevalent in the commercial market than fruity acidic variants (Table 1.1).

Table 1.1. Top 10 flavours of 250 commercially available ready-to-drink dairy protein-enriched beverages available worldwide (Mintel, 2018).

Flavour	% of Commercial Products
Unflavoured/Plain	45.8
Chocolate	19.3
Vanilla/Vanilla Bourbon/Vanilla Madagascar	10.2
Strawberry	6.10
Banana	4.50
Cocoa/Cacao	4.20
Malt	1.50
Raspberry	1.50
Mango	1.10
Coconut	1.10

Both acidic and neutral pH protein beverages are impacted by protein denaturation and aggregation mechanisms of milk proteins during thermal processing. This can have serious implications for the dairy industry in terms of operational costs associated with fouling (Britz and Robinson, 2008), and final product quality (Wijayanti *et al.*, 2014; Brodkorb *et al.*, 2016). These denaturation and aggregation mechanisms are dominated by heat-labile whey proteins, particularly β-lg due to its high concentration and reactivity in comparison to other proteins. At temperatures in excess of ~ 60 °C, whey proteins undergo conformational changes due to unfolding of their native compact globular structures, and subsequent aggregation with themselves or with κ-casein (Donato and Guyomarc'h, 2009; Wijayanti *et al.*, 2014; Chevallier *et al.*, 2016). However, separate denaturation temperatures have been determined for the different whey protein fractions (Table 1.2). The unfolding of globular proteins is an

endothermic process, while aggregation has been identified as an exothermic process by differential scanning calorimetry (DSC) (Singh and Havea, 2003; Fitzsimmons *et al.*, 2007). These denaturation and aggregation mechanisms occur in two distinct stages; firstly, unfolding of β-lg at denaturation temperatures, and secondly, the association of these unfolded molecules to form aggregates (Mulvihill and Donovan, 1987; Joyce *et al.*, 2017). As whey proteins unfold, they expose disulphide bonds, free sulfhydryl (-SH) and hydrophobic groups previously at the core of native protein molecule resulting in the formation of intramolecular disulphide bonds and increased hydrophobic interactions between molecules (Wijayanti *et al.*, 2014; Brodkorb *et al.*, 2016). Resulting sulfhydryl-disulphide interchange and hydrophobic interactions associate unfolded protein molecules with each other to form aggregates. The formation of protein aggregates leads to increases in particle size and viscosity in a dairy protein system (Brodkorb *et al.*, 2016).

Table 1.2. Thermal denaturation temperature and enthalpy of whey proteins (Brodkorb *et al.*, 2016)[1].

Protein	T_D °C	T_{tr} °C	ΔH kJ/mol
β-Lactoglobulin	78	83	311
α-Lactalbumin	62	68	253
Bovine serum albumin	64	70	803
Immunoglobulin	72	89	500

[1] T_D is the initial denaturation temperature, T_{tr} is the temperature at the differential scanning calorimetry (DSC) peak maximum, and ΔH is the enthalpy of denaturation.

The kinetics of protein denaturation have been investigated in order to understand factors influencing rates of heat-induced protein denaturation (Anema and McKenna, 1996; Kehoe *et al.*, 2011; Wolz and Kulozik, 2015). Wolz and Kulozik (2015) found

that denaturation of β-lg followed a 1.5 order reaction rate but that this rate was strongly dependent on temperature and protein concentration. Higher protein concentrations have been shown to affect the aggregation step, resulting in the formation of high molecular weight aggregates (Fitzsimons et al., 2007; Dissanayake *et al.*, 2013). Other factors can also influence the denaturation kinetics of whey protein such as the presence of casein (Brodkorb *et al.*, 2016). High proportions of casein in a dairy system can reduce the rate of protein denaturation and the quantity of heat-induced whey protein aggregates (Yüksel and Erdem, 2005; Anema *et al.*, 2006; Singh *et al.*, 2015). The presence of casein in a dairy system has been shown to limit irreversible aggregation during thermal processing and improve of the systems heat stability through a protective chaperone-like effect (O'Kennedy and Mounsey, 2006; Liyanaarachchi and Vasiljevic, 2018). Molecular chaperone proteins are involved in the transport, assembly, degradation and stabilisation of proteins as they recognise and bind to non-native states of protein that result from cellular stresses like heat, oxidation and reduction (Morgan et al. 2005). Casein possesses many properties similar to that of a molecular chaperone, providing hydrophobic surfaces to unfolding proteins in order to prevent irreversible aggregation as the result of thermal and non-thermal stress (O'Kennedy and Mounsey, 2006). This can be seen as κ-casein acts like molecular chaperone, blocking exposed hydrophobic surfaces of denaturing substrate proteins (Yong and Foegeding, 2008; Mounsey and O'Kennedy, 2010; Gaspard *et al.*, 2017).

In the production of acidic beverages, astringency and turbidity are significant issues that arise which impact consumer perception. Clear acidic beverages have been shown to be preferred by consumers compared to opaque variants (Beecher *et al.*, 2005). Increased turbidity in clear protein beverages is generally the result of denaturation and aggregation of whey proteins, causing turbid white precipitate (LaClair and Etzel,

2010). A number of methods to limit this denaturation and aggregation have been proposed, including the addition of compounds like food-grade lauryl sulfate (Etzel, 2004), the amino acid proline (Reddy *et al.*, 2005), urea, salts (LaClair and Etzel, 2010), and alcohols (Romero *et al.*, 2007). LaClair and Etzel (2010) proposed reducing turbidity by removing aggregates through centrifugation and altering pH.

Lee and Lawless (1991) described astringency as a group of complex sensations that involve the drying and perceived roughness of oral surfaces, with the additional perception of tightening, drawing in, or puckering of the oral mucosa and muscles around the mouth. It has been reported that astringency is associated with increased turbidity of beverage and protein-saliva mixtures, suggesting that interactions between salivary and whey proteins form aggregates related to astringency (Beecher *et al.*, 2008; Vardhanabhuti *et al.*, 2010; Ye *et al.*, 2011). Generally, low pH protein beverages with protein concentrations greater than 3% are considered highly astringent (Sano *et al.*, 2005; Beecher *et al.*, 2008). The astringency is often masked by the addition of sweeteners which can have negative connotations for health-conscious consumers seeking 'clean label' performance and sports beverages (LaClair and Etzel, 2010).

Ready-to-drink neutral pH beverages are often milk-based beverages enriched with dry powder ingredients like milk and whey protein concentrates (MPC and WPC, respectively) and isolates (MPI and WPI, respectively). Incorporation of these high protein powder ingredients can pose challenges in the beverage systems, as they commonly exhibit poor solubility (Jambrak *et al.*, 2008; Crowley *et al.*, 2014; Eshpari *et al.*, 2014). Solubility can be improved by the application of high temperatures, shear and increased hydration time (Pathania *et al.*, 2018). High heat treatment of dairy protein solutions can result in the promotion of off-flavours in the final product. 'Stale'

flavours result from increases in aldehyde compounds (Zabbia *et al.*, 2012), while 'cooked' flavours are caused by sulphur compounds connected to the exposure of free –SH groups during denaturation of β-lg (Al-Attabi *et al.*, 2009). Studies have investigated reducing the production of these off-flavours through the application of alternative treatment technologies which involve reduced thermal load on the product (Krešić *et al.*, 2008; Deeth and Lewis, 2016; Lee *et al.*, 2017).

Thermal stability of neutral pH beverages can be a technical challenge due to the high thermal processing requirements, particularly for formulations with a high proportion of heat-labile whey protein (Ryan and Foegeding, 2015). Heat-induced aggregation of whey proteins in a beverage system can result in increased turbidity and viscosity, phase-separation, precipitation and gelation, all of which can negatively impact product quality, shelf-life and consumer perception (Le *et al.*, 2016; Joyce *et al.*, 2017; Jiang *et al.*, 2018).

There is significant interest in developing methods for improving the heat stability of protein systems, particularly whey proteins (Wijayanti *et al.*, 2014). Recent studies have investigated pre-treatment of whey proteins to produce soluble aggregates that promote increased thermal stability of the product during final heating and storage (Ryan and Foegeding, 2015; Chevallier *et al.*, 2016; Joyce *et al.*, 2017; Wagoner and Foegeding, 2017). At lower temperatures, 60 – 70 °C, β-lg is seen to partially unfold into a molten globule-like state and result in some irreversible modification due to exposed thiol groups. While the application of higher temperatures results in increased unfolding followed by aggregation due to disulphide bond formation and hydrophobic interactions takes place (De Wit, 2008; Laiho et al., 2015). Different preheat temperature-time combinations at lower temperatures have been shown to alter the type of aggregation for heat-labile proteins, while the overall extent of whey protein

denaturation remains the same and can result in this enhanced thermal stability (Williams *et al.*, 2008). Enhanced thermal stability of whey proteins used in beverages has also been achieved through the application of technologies such as microparticulation (Dissanayke and Vasiljevic, 2009; Çakir-Fuller, 2015), and ultrasonication (Chandrapala *et al.*, 2011; Jambrak *et al.*, 2014; Nam *et al.*, 2017). Microparticulation involves the production of micro-aggregates with enhanced thermal stability through the application of high shear and pressure during thermal aggregation of protein (Renard *et al.*, 2002; Çakir-Fuller, 2015). Ultrasonication is the application of soundwaves to liquid food resulting in pressure differential cycles to create high localised turbulence, shear, pressure and temperature (McCarthy *et al.*, 2016).

1.5. Forms of heat treatment

Heat treatment is applied to raw milk and its derivatives in order to render the products safe for consumption, and to extend the shelf life. It is a critical control point or CCP in terms of the microbial quality of milk products. The aim of heat treatment processes is to reduce the pathogenic and spoilage microbial populations, and inactive enzymes, while minimising chemical reactions and physical changes in the product (Lewis and Deeth, 2009). There are numerous thermal processing technologies, temperature-time combinations, and stabilisation strategies, which can be employed in combination in order to achieve desired properties of a final product.

The severity of heat treatment applied to a product can be selected according to the properties required for the final product. There are a number of heat treatment severity classifications related to general heat treatment temperature-time combinations which are commonly used. Thermisation, low-temperature long-time (LTLT) pasteurisation,

high-temperature short-time (HTST) pasteurisation, extended shelf life (ESL) treatment, ultra-high temperature (UHT) processing and in-container sterilisation are each long-established heat treatment classifications for dairy products (Burton, 1994). Each of these classifications are associated with typical temperature-time combinations and result in different levels of microbial and chemical change and length of shelf-life (Table 1.3).

Table 1.3. Typical temperature-time combinations associated with common heat treatment classifications (Fox *et al.,* 2015).

Classification	Temperature-Time Combination
Thermisation	65 °C x 15s
Pasteurisation	
LTLT (low temperature, long time)	63 °C x 30 min
HTST (high temperature, short time)	72 °C x 15 s
Fore warming for sterilisation	90 °C x 2 – 10 min
	120 °C x 2 min
Sterilisation	
UHT (Ultra-high temperature)	130 – 140 °C x 3 – 5 s
In-container	110 – 115 °C x 10 – 20 min

Thermisation is a mild form of heat treatment, which has a minimal effect on the chemical properties of the dairy product. It is usually followed by further heat treatment before consumer consumption. The primary aim of this heat treatment is to reduce the growth of temperature-sensitive microorganisms (3 - 4 log reduction), such as psychotrophic bacteria which can release heat-resistant protease and lipase enzymes into the milk, which can result in off-flavours and age gelation in milk products

(Lewis, 2003; Britz and Robinson, 2008; Fox *et al.*, 2015). Typical conditions for thermisation can range from 57 - 68 °C for 15s, and generally relates to a shelf life of 3 days at a maximum of 8 °C (Lewis and Deeth, 2009).

Tyndallisation, also known as intermittent or fractional sterilisation, is another milk heat treatment classification. The process involves three days of successive heat treatments, at 100 °C for 3 min, followed by storage at ambient temperature (Tyndall, 1877, Brown *et al.*, 1979). The aim of the treatment is to target spores by allowing spore germination between heat treatments and subsequent inactivation by the following heat treatment. However, due to the unpredictability of spore germination, the process is not commonly used (Lewis, 2003). The most recent research application of tyndallisation was for Korean rice beverages, which applied a modified form of tyndallisation in conjunction with CO_2 injection (Kim *et al.*, 2012).

Pasteurisation is a widely used heat treatment which can be carried out in batch, LTLT (60 °C for 30 min), or continuous, HTST (72 °C for 15 s), flow operations. The treatment reduces levels of all vegetative pathogenic microorganisms, with minimal chemical, physical or organoleptic changes (Rysstad and Kolstad, 2006; Lewis and Deeth, 2009). Pasteurised products generally last up to 48 hr without refrigeration and for several days when refrigerated (Lewis, 2003). However, consumer demands for longer life milk and improvements in processing technologies and post-processing contamination (PPC) control have enabled commercial processing plants to achieve longer shelf life for HTST pasteurised milk, of 17 – 21 days (Barbano, 2017).

ESL, also known as ultra-pasteurisation (UP), treatment involves the reduction of a products microbial count beyond that achieved by normal pasteurisation treatment. An agreed definition for ESL does not exist; however, the temperatures typically

employed are greater than that of pasteurisation for a shorter period of time, e.g. 125 – 135 °C for 2-4 s, and heat treatment is followed by packaging under aseptic conditions (Rysstad and Kolstad, 2006). This heating stage can be combined with membrane filtration in order to improve shelf life further (Lorenzen *et al.*, 2011). The application of microfiltration without heat treatment has also been applied to extend the shelf-life of dairy products (Hoffmann *et al.*, 2006). ESL treatment can increase the shelf life of a milk product to between 30 and 90 days, depending on the type of ESL treatment applied (Bertolini *et al.*, 2016). The production of ESL treated milk products is increasing to meet consumer and supply chain requirements in countries like the United States (Lee *et al.*, 2017).

UHT treatment is a severe form of heat treatment with typical temperature-time combinations of 135 – 150 °C for 2-4 s, followed by aseptic packaging. This treatment significantly reduces the microbial population of the product, extending the product shelf-life to between six months and a year under ambient conditions (Malmgren *et al.*, 2017). The long shelf life has led to an increase in the consumption of UHT milk worldwide, particularly where distance and cold-chain refrigeration make storage problematic. However, the high temperature results in noticeable chemical changes with regard to flavour and colour, with many consumers perceiving “cooked” or “stale” flavours (Al-Attabi *et al.*, 2009; Barbosa-Cánovas and Juliano, 2008; Zabbia *et al.*, 2012; Jansson *et al.*, 2014; Dursun *et al.*, 2017). There is significant research being carried out to improve the quality of UHT products by investigating improvements in product stability (Chen *et al.*, 2015; Deeth and Lewis, 2016), thermal processing technology (Browning *et al.*, 2001; Tewari and Juneja, 2007; Lee *et al.*, 2017; Malmgren *et al.*, 2017), and the application of pre- and final heat temperature-time combinations (Srichantra, 2006).

In-container sterilisation is the most severe form of heat treatment classification due to the relatively high temperatures and long processing times employed, with typical temperature-time combinations of 105 - 120 °C for 10 - 30 min being applied to the product in a sealed container (Burton, 1994). The final product is rendered commercially sterile as all vegetative microorganisms are destroyed or incapable of growth and target spoilage rates are less than 1 in 10,000 containers. The treatment can be carried out in batch operation or using continuous retorting processes (Deeth and Lewis, 2016). There are many products, such as evaporated or sweetened condensed milk, still being produced in this manner and research is still on-going in the area (Chen *et al.*, 2015; Crowley *et al.*, 2015; Deeth and Lewis, 2015; Chen and O'Mahony, 2016; Dumpler *et al.*, 2017).

1.6. Thermal processing technologies

Several types of heat exchanger are used in food processing to accommodate diverse requirements of cooling, heating and sterilising for various food products (Saravacos and Kostaropoulos, 2016). There are two general categories of thermal processing technology; direct and indirect heating. Indirect heating systems involve heat being transferred from one fluid to another across a thermally conducting, but impermeable, interface (Hsu, 1970; Roux *et al.*, 2016). The integrity of this barrier is a critical control point with regard to product safety and quality (Lewis and Deeth, 2009). Direct heating systems do not contain a barrier, but instead the product and heating fluid, generally steam under pressure, are mixed directly resulting in rapid heating and is subsequently removed through flash cooling (Datta *et al.*, 2002; Rauh *et al.*, 2014).

1.6.1. *Indirect heating technology*

Indirect heat-exchangers are the most widely used type of thermal processing technology. They are versatile, available for both heating and cooling operations, and enable the reduction in energy consumption through heat regeneration and pinch point analysis (Fraas, 1989). Indirect heat exchangers are available in a number of forms, for example, plate heat exchangers (PHE), tubular heat exchangers (THE) and scraped surface heat exchangers (SSHE).

PHEs are well-established as a continuous-flow thermal heating technology and are widely used for the pasteurisation of milk, cream, ice-cream, fruit juices and similar products (Lewis *et al.*, 2000). These exchangers consist of thin metal plates pressed together to form channels through patterns of turbulence-promoting corrugations that define heating and cooling flow passages (Fig. 1.2). The product and heating medium streams are separated alternately by plates, through which the heat transfer process can take place for any combination of gas, liquid and two-phase streams. Gaskets, which fit into groves pressed into the plate surface, seal the joints between the outer edges of each plate and the fluid inlet and outlet manifolds, in order to prevent contamination of treated product with untreated product or heating fluid. The plates are forced together by long tie rods and bolts at the periphery to provide a good seal at these gasketed joints and carry the pressure loads required. Forms of PHE other than the gasketed plate type described here also exist, such as the spiral plate or lamella (Fraas, 1989, Kakac *et al.*, 2012).

PHEs can achieve high heat transfer rates per unit heat transfer area, despite their compact size, as the corrugations can be designed to give high levels of turbulence in the flowing stream with a small channel size. The systems are also flexible whereby several heat exchanger sections performing different duties (e.g., heating, cooling and

regeneration) can be fitted into a single frame assembly with suitable interconnections. Routine cleaning is carried out using cleaning-in-place (CIP) systems, which are, in general, capable of removing any fouled material from heated product. However, the plates can be easily separated and cleaned manually if extensive cleaning or inspection is required (Lewis *et al.*, 2000; Abu-Khader, 2012). PHEs do carry a risk of heat treated product contamination due to the thinness of the plates, for example pinhole failures, and the use of seals to separate material. However, regular maintenance can generally avoid such complications (Shah and Sekulic, 2003).

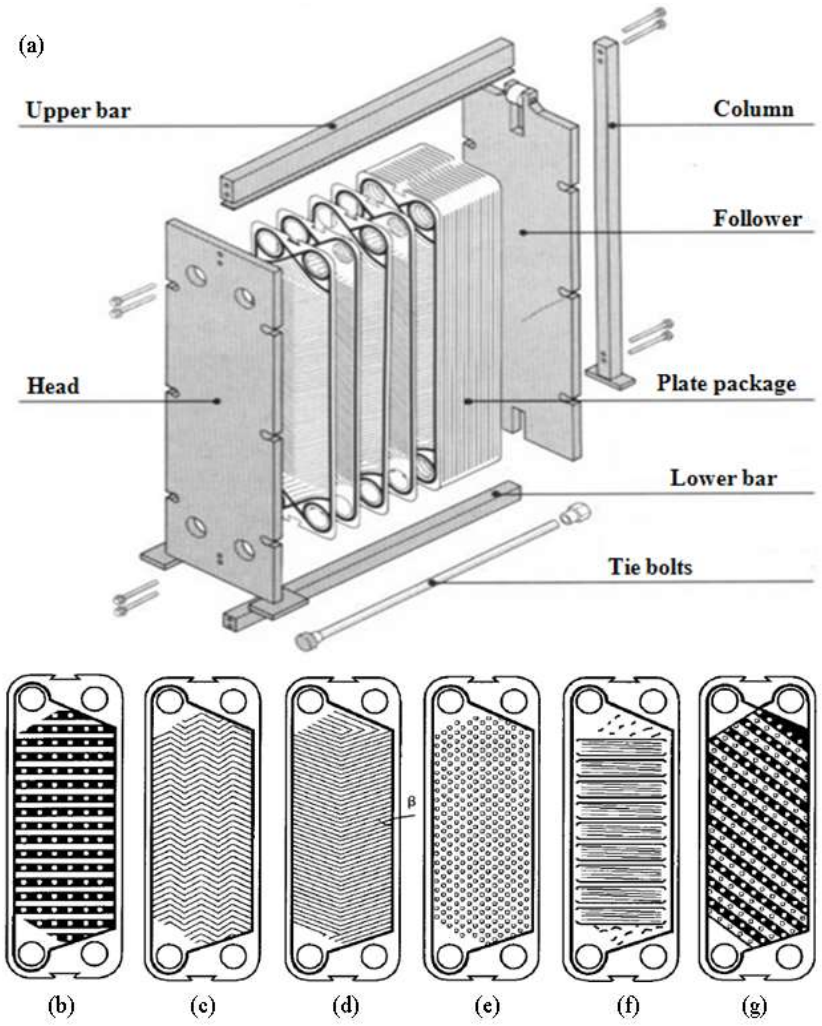

Fig. 1.2. Overall design of (a) a plate heat exchanger and some typical plate corrugation patterns; (b) washboard, (c) zigzag, (d) chevron or herringbone, (e) protrusions and depressions, (f) washboard with secondary corrugations, and (g) oblique washboard (Mota *et al.,* 2015).

Limiting protein denaturation and fouling during thermal processing with PHEs has significant industrial relevance as a result of the significant economic costs associated with the issue (Bansal and Chen, 2006). Many recent studies are related to the investigation of fouling (Petit *et al.*, 2013; Boxler *et al.*, 2014a; Boxler *et al.*, 2014b; Khaldi *et al.*, 2015; Blanpain-Avet *et al.*, 2016), cleaning (Memisi *et al.*, 2015), and thermal efficiency (Taghizadeh-Tabari *et al.*, 2016) in PHE's processing dairy products. Computational fluid dynamics (CFD) calculations have been applied to model denaturation and aggregation of β-lg in a PHE (Bouvier *et al.* 2014; Rios-Iribe *et al.*, 2016; Luan *et al.*, 2017).

THEs consist of circular tubes in which one fluid, either product or heating medium, flows inside the tubes and the other flows outside. Heat transfer occurs across the thermally conducting tube wall. The exchanger heat transfer efficiency can be optimised by changing the tube diameter, length, pitch, arrangement and the number of the tubes, allowing a degree of flexibility in design. The risk of contamination of heat treated product is relatively low within THE systems, due to the mechanical strength and resistance to corrosion of the stainless-steel tubes used (Kakac *et al.*, 2012).

There are two main classifications of THE used in the food industry: concentric tube (or double tube) and shell-and-tube heat exchangers (Fig. 1.3). Shell and tube heat exchangers consist of a large pressure vessel or shell, with bundles of tubes inside. The concentric tube systems consist of tubes of stainless steel assembled one inside the other with a spacer, generally a spiral wire, in each inter-tube space to maintain the tubes as concentric. This tube is then coiled and housed within an outer cylindrical unit for hygienic and mechanical protection. Double-tube systems consist of two concentric tubes, where the product flows in the centre tube and the heating or cooling

medium flows through the space between and tend to be used for simple heating or cooling. Triple-tube systems are used for intensive heating requirements such as final stage heating to UHT or sterilisation temperatures and consist of one product flow tube between two tubes through which the heating or cooling medium flows, as required. This tube arrangement provides and increased heat transfer area and therefore improved heat transfer coefficient, compared to the double-tube system (Burton, 1994; Lewis *et al.*, 2000; Saravacos and Kostaropoulos, 2016).

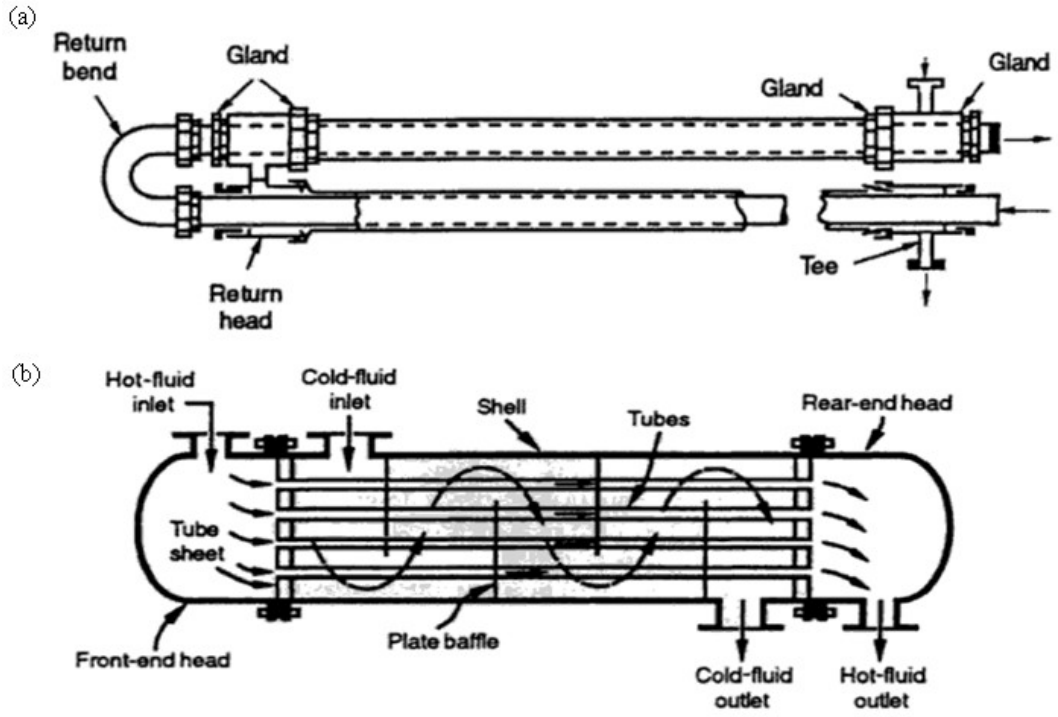

Fig. 1.3. Overall design of a (a) concentric tubular heat exchanger and (b) shell-and-tube heat exchanger (Shah and Sekulic, 2003).

THEs have a larger flow channel compared to PHEs and as a result, they are capable of processing products which have a higher viscosity, or which contain high levels of pulp, fibres, or even particulate solids, with a reduced level of fouling. However, due to this larger flow channel the effective heat transfer area is lower in relation to the volume of liquid, resulting in a lower heat transfer rate compared to that achieved by

a PHE (Lewis *et al.*, 2000). As for PHEs, there is significant interest in fouling mechanisms associated with THEs during dairy processing and developing methodologies to limit this fouling (Petermeier *et al.*, 2002; Huertas *et al.*, 2015; Prakash *et al.*, 2015; Phinney *et al.*, 2017). Kerche *et al.* (2016) investigated the aggregation mechanisms of microparticulated whey protein in a tubular heat exchanger.

Scraped surface heat exchangers (SSHE) consist of a cylindrical vessel, in which the product is placed, and an outer jacket, containing a heating or cooling fluid as required (Fig. 1.4). A shaft, at the vessel's central axis, supports scraper blades and rotates within the vessel, continuously scraping product from the heat transfer area of the cylinder, returning product to the bulk and gradually moving the product to the outlet (Lewis *et al.*, 2000; Dehkordi *et al.*, 2015). Such exchangers are generally used for highly viscous or sticky products, or that contain particles and tend to foul a heat transfer surface with deposits and films (Lewis *et al.*, 2000; Rao and Hartel, 2006).

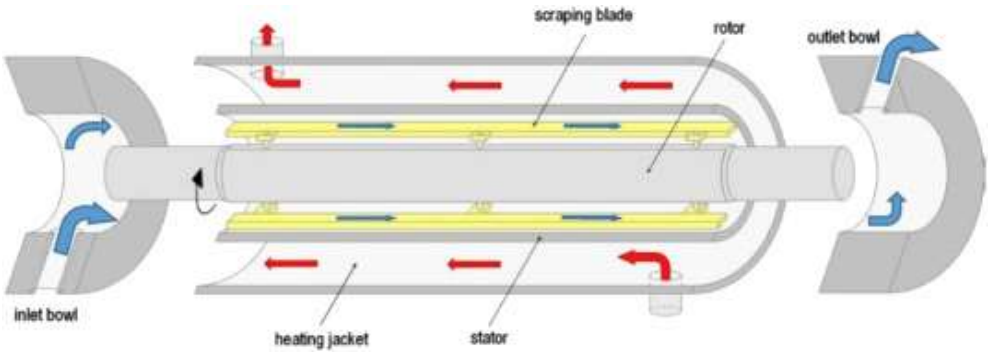

Fig. 1.4. Overall design of a scraped-surface heat exchanger (Dehkordi *et al.,* 2015).

Spiegel (1999) found that the size and structure of whey protein aggregates could be influenced by the level of shear in SSHE technology during heating of whey protein concentrate solutions (10% protein (w/w)). In the dairy industry, SSHEs are more often used in the production of frozen products such as ice cream (Rao and Hartel,

2006; Ali and Baccar, 2017; Parra *et al.*, 2018), while little has been published recently on SSHE thermal treatment in a dairy space.

1.6.2. *Direct heating technology*

Direct heating technologies were first developed and investigated over half a century ago (Brown *et al.*, 1951; Ford *et al.*, 1969; Patrick and Swaisgood, 1976; Bassette and Jeon, 1983), however, there has been renewed interest in the technology, particularly steam injection, over the last 10 years (Fig. 1.5). This may be in response to consumer demand for convenient products with a longer shelf-life which retain their natural flavours (Lewis and Deeth, 2009).

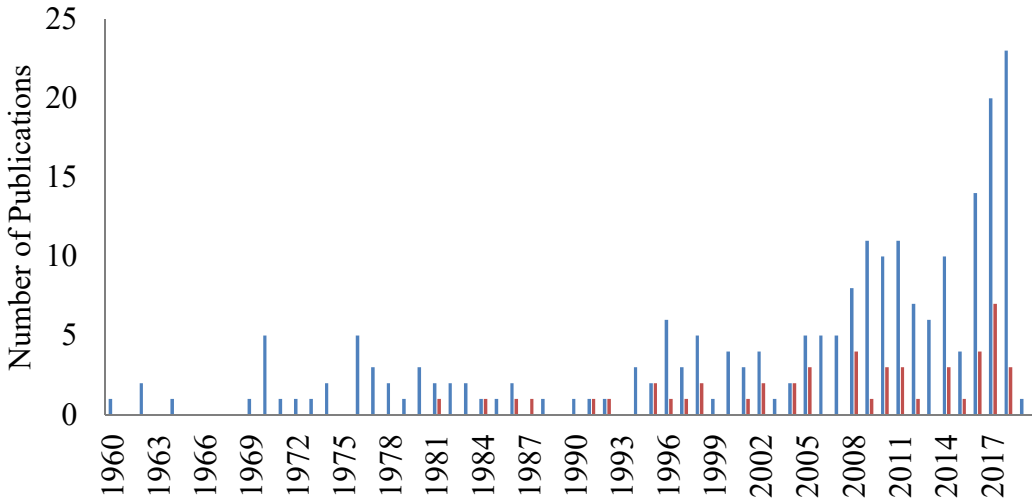

Fig. 1.5. The number of peer-reviewed publications each year for direct steam injection (■) and direct steam infusion (■) under the search query "direct steam injection milk", "direct steam injection dairy", "direct steam infusion milk", and "direct steam infusion dairy" (Scopus, 2018).

Studies investigating the application of direct heating technologies encompass a wide range of different food products such as milk (Rauh *et al.*, 2014; Akkerman *et al.*, 2016; Troung et al., 2017; Lee *et al.*, 2017; Jo *et al.*, 2018), WPC solutions (Dickow *et al.*, 2012a; Dickow *et al.*, 2012b), concentrated milks (Dumpler and Kulozik, 2016;

Dumpler *et al.*, 2017; Dumpler *et al.*, 2018), infant formula (Murphy *et al.*, 2011; Murphy *et al.*, 2013, Roux *et al.*, 2016), pea and rice protein isolate solutions (Pietrysiak *et al.*, 2018), tomato concentrate (Casoli, 2013), white radish broth (Ham and Yoon, 2017), orange peel waste (Santi *et al.*, 2015) and even squid protein hydrolysates (Karayannakidis *et al.*, 2014).

Direct heating is generally carried out by one of two systems; direct steam injection and direct steam infusion. For steam injection systems (steam into milk), the superheated steam is added in-line to the product, while in steam infusion systems (milk into steam), the product is passed through a steam-filled chamber as a thin film or fine streams (Datta *et al.*, 2002; Lee *et al.*, 2017; Fig. 1.6). Both modes of operation result in an almost instantaneous rise in the temperature of the product, during which the steam in condensed and the milk is diluted with water. This entrained water is removed through flash cooling, applied after any required holding tube, which also facilitates rapid cooling of the product (Lewis and Deeth, 2009). The quantity of steam added is dependent on the product flowrate and temperature increase required. Lewis and Heppell (2002) reported that for a 60 °C increase in temperature, for example heating milk from 80 to 140 °C, a liquid volume increase of approximately 11 % is required.

Alternative versions of conventional steam injection and infusion systems have been proposed. Dickow *et al.* (2012a) investigated the application of lenient steam injection (LSI), a patented heating process which uses direct heat transfer. In the LSI system, steam is mixed into the product steam using a dynamic mixer, ensuring the formation of smaller bubbles and enabling faster heat transfer (Westergaard, 2004; Dickow *et al.*, 2012a; Dickow *et al.*, 2012b). Studies showed that LSI produced low levels of whey protein denaturation (30 - 35 %) in whey protein concentrate solutions with 23

% protein (w/w) at 90 °C (Dickow *et al.*, 2012b). However, studies were not conducted comparing LSI to conventional steam injection or infusion, so it is difficult to quantify the advantages gained through this alternative design.

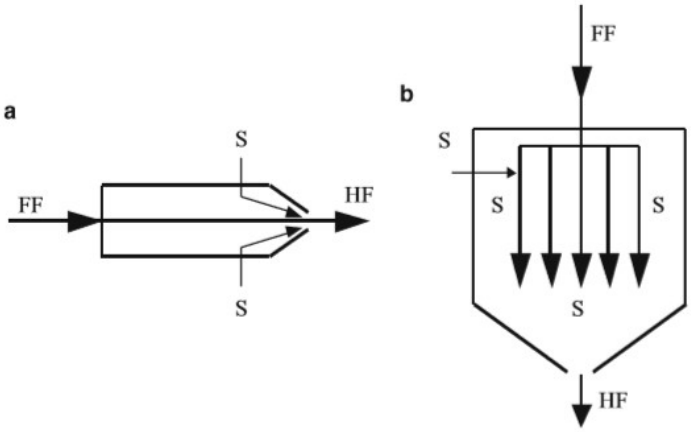

Fig. 1.6. Diagram of direct steam (a) injection and (b) infusion heat treatment technologies, where FF indicates fluid food, HF indicates heated food and S indicates steam (Saravacos and Kostaropoulos, 2016).

There are technical and economic challenges with direct systems such as the requirement for culinary-grade steam, lower heat regeneration capacity, and concerns with product dilution, resulting in indirect technologies being more commonly used industrially (Datta *et al.*, 2002; Britz and Robinson, 2008; Dickow *et al.*, 2012a; Karayannakidis *et al.*, 2014; Lee *et al.*, 2017). Operation of the flash cooler is an important control step to limit dilution or concentration of the product, involving the maintenance equal temperatures at the preheating and flash cooling stages. However, in practice heat losses within the system, the amount of moisture in the steam and the equipment design may alter the level of water entrained in the product (Datta *et al.*, 2002). The total solids of the product should be monitored for dilution or concentration throughout production and the temperature of the flash cooler adjusted if required.

Dilution of product is commonly reported in pilot-scale operation of direct heating systems (Dickow *et al.*, 2012b; Murphy *et al.*, 2013; Dumpler *et al.*, 2017).

Direct heating is a more thermally efficient mode of heating as the latent heat of vaporisation, generally lost during conventional indirect treatments, is given up to the product as the steam condenses (Lewis, 2000; Britz and Robinson, 2008). A lower thermal load is imparted on product, compared to indirect heating, due the rapid heating and cooling operations resulting in a reduced residence time (Fig. 1.7). Due to the significant differences in heating mechanisms and temperature-time profiles of direct and indirect heating systems can impact the characteristics of heat-treated products.

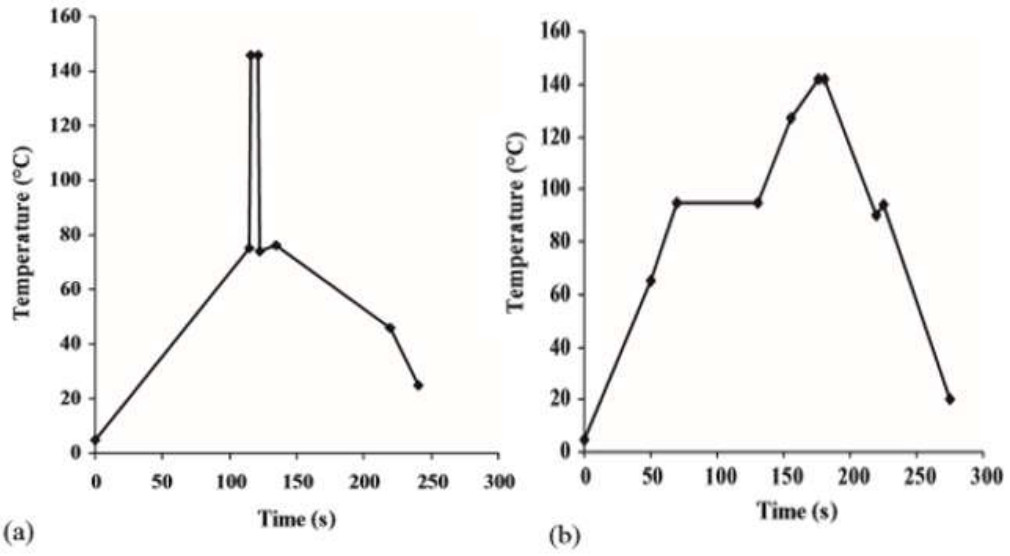

Fig. 1.7. Temperature-time profiles of typical (a) direct and (b) indirect UHT heat treatment plants, indicating the thermal load imparted on the product (Lewis and Deeth, 2009).

As a result of the lower thermal load, studies have found the application of direct heat treatment has resulted in significantly reduced whey protein denaturation compared to direct heating in skim milk (Akkerman *et al.*, 2016; Lee *et al.*, 2017) and whey protein

concentrate systems (Dickow *et al.*, 2012b). Akkerman *et al.* (2016) reported steam injection-treated skim milk with β-lg A and B denaturation levels of 24.6 and 15.8 %, respectively, compared to more than 90 % denaturation of β-lg A and B for skim milk heated using plate and tubular heating technologies, when heat treated at 115 °C for 4 s.

Directly-heated milks are reported to have fewer volatile compounds associated with 'off-flavours' than indirectly-treated milks. The rapid cooling achieved by the flash cooling operation in direct heating systems has been shown to remove dissolved oxygen, heat-induced sulphur volatiles and other volatiles, in addition to removing excess water, resulting in less heat-induced flavour changes (Deeth and Lewis, 2016; Lee *et al.*, 2017). Lewis and Deeth (2009) reported that the oxygen level in directly-treated milks can be < 1 µL/mL, compared to 7-9 µL/mL found in indirectly-treated milks. Dissolved oxygen in UHT milk can result in the production of oxidised flavour and vitamin destruction over storage (Adhikari and Singhal, 1992). Concentrations of dissolved oxygen in excess of 7 ppm rapidly oxidise free -SH groups result in a reduction of cooked flavour, but increasing the appearance of oxidised, stale flavours (Al-Attabi *et al.*, 2009; Al-Attabi *et al.*, 2014). The reduced level of volatile compounds is also related to the lower thermal load imparted by direct systems and the associated reduced level of protein denaturation. Denaturation of β-lg is the main source of –SH compounds and the associated cooked flavour in milk, so that milks with a greater degree of protein denaturation have a distinctly more cooked flavour, negatively impacting consumer perception (Walstra *et al.*, 1999; Al-Attabi *et al.*, 2009).

There is less fouling in direct heat treatment systems compared to indirect systems as there is no interface between the heating medium and product where localised heating,

protein denaturation and fouling typically take place (Murphy, 2011; Karayannakidis *et al.*, 2014; Akkerman *et al.*, 2016). Truong *et al.* (2017) found no significant fouling in a direct steam injection system after 8 hrs of processing whole milk at both 80 and 120 kg/hr with temperatures of 75 °C preheat and 95 °C final heat applied. To induce fouling for their study, they used sudden expansion test pieces, featuring an instant increase in internal diameter from 10 to 23 mm, in the pipe downstream of the injector which disturbed the flow and initiated fouling. When induced, they found that the fouled material had high levels of protein, particularly β-lg, and fat. Studies have reported direct heat treatment of dairy systems can result in a larger particle size and increase in sediment formation during storage compared to indirect heating (Burton, 1968; Datta *et al.*, 2002). It has been suggested that this is related to the reduced in fouling in direct systems, where highly aggregated material, which would generally adhere to the hot surfaces of indirect systems, does not deposit but instead remains within the product stream and final product (Malmgren *et al.*, 2017).

1.6.3. *Alternative thermal and non-thermal technology*

There are a number of alternative thermal and non-thermal treatment technologies available with the capability of rendering food microbiologically safe and extending shelf life. Many of these alternative technologies purport to accomplish this while minimising degradation of food quality and sensory attributes, making them attractive options for processors looking to deliver products to meet minimal processed requirements increasingly demanded by consumers.

Ohmic heating (OH), is based on the principle that most food products resist the passage of electric current. By applying an alternating electrical current through the food, heat is generated within the food itself due to this electrical resistance (Pereira and Vicente, 2010). The technology has long been available and has researched since

the end of the 19th century (Jones, 1897; Jager *et al.*, 2016). There are a number of advantages to the use of OH compared to conventional heating methods. Like direct heating methods, OH avoids the thermally inefficient mechanism of conduction of heat through a surface into the product (Bansal and Chen, 2006). As there are no hot heat transfer surfaces in the OH system, fouling issues are greatly reduced compared to conventional heat treatment (Stirling, 2009). There have been a number of studies investigating the application of OH to dairy systems such as milk (Bansal and Chen, 2006; Stanel and Zitney, 2010; Kim *et al.*, 2017), whey solution (Icier, 2009), and infant formula (Roux *et al.*, 2016; Courel *et al.*, 2011). Roux *et al.* (2016) compared the thermal impact of OH and direct steam injection on liquid infant formula and found that both technologies produced infant formula of equivalent quality in terms of soluble protein, vitamin C, Maillard reaction products, and colour. Comprehensive reviews on OH have been conducted by Jaeger *et al.* (2016) and Cappato *et al.* (2017).

Pulsed electric field (PEF) processing is the application of short micro second pulses of high electric fields with an intensity of 20 – 80 kV/cm via two electrodes, with the processing time equal to the effective pulse duration i.e. pulse duration multiplied by number of pulses (Pal, 2017; Buckow *et al.*, 2014). Like OH, PEF is a non-thermal processing technique is reported to maintain the sensory and nutritional attributes of milk, while extending the shelf life (Lasekan *et al.*, 2017). There have been a number of studies undertaken to investigate the application of PEF to dairy systems (Craven *et al.*, 2008; Walkling-Ribeiro *et al.*, 2009; Sharma *et al.*, 2014a; Sharma *et al.*, 2014b), however, many of these studies focus on the microbial inactivation of milk rather than the physical characteristics of PEF-treated milks. Bendicho *et al.* (2002) found that pasteurisation of milk with PEF technology resulted in minimal changes to water- and fat-soluble vitamins, while still achieving the required microbial inactivation. PEF can

also be combined with other preservation technologies like low-temperature conventional heat treatment, microfiltration (> 1.2 μm pores size), and ultra-violet irradiation, to improve the preservation effect (Lasekan *et al.*, 2017). Buckow *et al.* (2014) have published a comprehensive review on the application of PEF in a dairy space.

High pressure processing (HPP), also referred to as high hydrostatic pressure (HHP) or ultra-high pressure (UHP) processing, is a non-thermal food preservation technology involving the application of 100 to 800 MPa of pressure to foods in a vessel specifically designed to withstand these pressures. A liquid, typically water, is used as a pressure transfer medium which surrounds the food and imparts an even pressure on the food (Huang *et al.*, 2017). The treatment is generally carried out at room temperatures, but can be specified at temperatures between 0 to 100 °C. The exposure time are generally short but can also be varied, ranging from a millisecond pulse to 20 min (Farkas and Hoover, 2000). The application of HPP can destroy micro-organisms, without significant changes to the sensory or nutritional properties of dairy products due to thermal degradation. HPP is one of the most successfully commercialised non-thermal processing techniques, applied to a wide range of food products (Huang *et al.*, 2017). Studies into the application of HPP were first conducted in the United States in 1885 and the first commercially available HPP-treated foods were in Japan in 1990 and featured high-acid fruit jams (Datta and Deeth, 1999). Today juices and ready-to-eat meat products are the most widely available types of HPP-treated products (Huang *et al.*, 2017). There has been little commercial use of HPP treatment in a dairy space as difficulty destroying bacterial spores, changes to the casein micelle structure above 300 mPa, and denaturation of β-lg above 100 MPa pose significant technical challenges (Datta and Deeth, 1999; Huppertz *et al.*, 2002; Cadesky *et al.*, 2017). The

combination of low thermal treatment with HPP technology was shown to significantly increase spore inactivation by 3.5 log for milk treated with 600 mPa for 40 min at 70 °C compared to 38°C (Silvia, 2015). High pressure-thermal processing (HPTP) which utilises HPP in combination with heating above 60 °C has also been applied to skim milk, however, this resulted in increased colour change and proteolysis compared to thermal treatments at atmospheric pressure (Devi *et al.*, 2015). A number of investigations have been recently completed regarding the application of HPP to human milk (Mateos-Vivas *et al.*, 2015; Contador *et al.*, 2015; Sousa *et al.*, 2016).

Continuous flow microwave heating (MWH) is an alternative method of heating which is reported to reduce thermal damage of treated food compared to conventional heat treatment. This is attributed to the rapid temperature rise and lack of hot surfaces in microwave heating (Lopez-Fandino *et al.*, 1996). The first study on the use of MWH for milk pasteurisation was in 1969 (Villamiel *et al.*, 1996). Microwaves are part of the electromagnetic spectrum with frequencies ranging from 300 MHz to 3 GHz, providing direct volumetric heating in foods from the conversion of electromagnetic energy (Coronel *et al.* 2003). Studies have shown that the levels of whey protein denaturation (Lopez-Fandino *et al.*, 1996; Villamiel *et al.*, 1996) and sensory attributes in MWH-treated milks (Valero *et al.*, 1999; Valero *et al.*, 2000; Clare *et al.*, 2005) are comparable to those achieved through conventional heat treatments. Sierra *et al.* (1999) showed that the application of MWH-treatment reduced the loss of vitamin B_1 compared to indirect treatment with a PHE. MWH treatment has been successfully applied during peptic hydrolysis of 1% whey protein solutions (w/v) to produce milk peptides with low allergenicity (El Mecherfi *et al.*, 2015). However, there have not been a great deal of recent studies conducted into the application of MWH to milk products.

1.7. Applying mathematical modelling to the thermally-induced changes in protein-enriched beverages

Appling mathematical modelling to the changes in physical characteristics observed during thermal processing of milk can provide significant insight into the mechanisms of these changes (Steffe, 1996). Mathematical modelling has long been applied to a wide range of factors influencing the quality of dairy products; protein denaturation and aggregation mechanisms, increases in viscosity, colour changes, microbiological inactivation, and shelf-life prediction. The application of mathematical modelling to viscosity in dairy systems is described in detail.

Thermo-physical properties of food, like viscosity, have significant impact on the processing, preservation and quality control of a product. O'Callaghan and Cunningham (2005) noted that advances in process control for dairy systems would involve the measurement of viscosity as a function of total solids, temperature, time and shear rate. Viscosity, a fluid flow property, which also has implications in heat transfer calculations, impacts on selection of equipment type, the calculation of equipment size and the design of processing control systems (Alcântara *et al.*, 2012; Messaâdi *et al.*, 2015; Saravacos and Kostaropoulos, 2016; Peleg, 2017). Viscosity is primarily influenced by temperature and pressure, typically altered as part of the thermal processing operation (Haj-Kacem *et al.*, 2014). Viscosity is a result of internal friction in a fluid and resistance to flow, as measured by the force per unit area resisting uniform flow ((N.s)/m2 or Pa.s) (Rao, 2013). This can be expressed as the equation:

$$\frac{F}{A} = \mu \frac{dv}{dx} \qquad 1.1$$

where μ is viscosity (Pa.s), F/A is force divided by area also known as shear stress (N/m2 or Pa), and dv/dx is the velocity gradient of the fluid known as shear rate (1/s).

Therefore, the equation for viscosity can be simplified in terms of shear stress (σ) and shear rate ($\dot{\gamma}$):

$$\mu = \frac{\sigma}{\dot{\gamma}} \quad 1.2$$

At a micro level, a fluid's viscosity arises from collision of particles and the force fields that determine interactions among molecules (Haj-Kacem *et al.*, 2014). Because of its impact on processing conditions, rheological characterisation is of critical importance in optimising food formulation and processing systems. The viscosity of a fluid is often expressed in terms of apparent viscosity which is a single viscosity measurement taken at a constant shear rate.

For over one hundred years, various forms of mathematical model (equations) have been applied to characterise rheological data of materials, such as liquid food products, in terms of temperature and concentration. The application of such models to the rheological behaviour of food systems allows complex rheological data to be described using a small number of parameters based on these mathematical equations (Steffe, 1996, Rao, 2013). Rheological models may be grouped into three categories; empirical, theoretical and structural, where empirical models are deduced from experimental data, theoretical models are based on fundamental physical concepts, and structural models are rooted in structure and kinetics combined with experimental data (Rao and Hartel, 2006; Rao, 2013). A wide range of models have been developed over the years describing the relationship between shear rate and shear stress, temperature and viscosity, concentration or volume fraction and viscosity, and many other rheological relationships (Table 1.4).

Table 1.4. Models describing Shear Rate ($\dot{\gamma}$) versus Shear Stress (σ) data (Rao, 2013, Saravacos and Kostaropoulos, 2016).

Formula[1]	Description
$\boldsymbol{\sigma = \mu\dot{\gamma}}$	Newtonian model
$\boldsymbol{\sigma = \sigma_o + \eta\dot{\gamma}}$	Bingham plastic model
$\boldsymbol{\sigma = K\dot{\gamma}^n}$	Power law model
$\boldsymbol{\sigma = \sigma_0 + K\dot{\gamma}^n}$	Herschel-Buckley
$\boldsymbol{\sigma = \dfrac{\dot{\gamma}}{\left[\dfrac{1}{\mu_0} + K_E\sigma^{(1/n_E)^{-1}}\right]}}$	Ellis model for low shear rate data containing η_0
$\boldsymbol{\sigma = [\mu_\infty\dot{\gamma} + K_s\dot{\gamma}^{n_s}]}$	Sisko model for high-shear rate data containing η_∞
$\boldsymbol{\mu_a = \mu_\infty + \dfrac{\mu_0 - \mu_\infty}{1 + (\alpha_c\dot{\gamma})^m}}$	Cross model for data over a wide range of shear rates
$\boldsymbol{\mu_a = \mu_\infty + \dfrac{\mu_0 - \eta_\infty}{\left[1 + (\lambda_c\dot{\gamma})^2\right]^N}}$	Carreau model for data over a wide range of shear rates
$\boldsymbol{\sigma^{0.5} = K_{0c} + K_c(\dot{\gamma})^{0.5}}$	Casson model used for chocolates
$\boldsymbol{\sigma^{0.5} - \sigma_{0M} = K_M\dot{\gamma}^{nM}}$	Mizrahi and Berk (1972) model is a modification on the Casson model
$\boldsymbol{\sigma^{n_1} = \sigma_0^{n_1} + \mu_\infty(\dot{\gamma})^{n_2}}$	Generalised model of Ofoli *et al.* (1987)
$\boldsymbol{\sigma = \left[(\sigma_{oV})^{1/n_V} + K_V\dot{\gamma}\right]^{n_V}}$	Vocadio model

Milk products are subject to a range of temperature conditions during the processing and as viscosity of all liquid products is significantly influenced by temperature, understanding the effect of these temperature changes on product viscosity is essential for design of thermal processing operations (Rao, 2013; Singh and Heldman, 2014).

Because of the role of molecular interactions in viscosity, most liquid foods exhibit some kind of inverse temperature-viscosity relationship, but in addition to such instantaneous effects of temperature on viscosity, products containing dairy proteins are subject to the effects of denaturation and aggregation of heat-labile proteins, (Mulvihill and Donovan, 1987; Fitzsimons *et al.*, 2007; Brodkorb *et al.*, 2016; Chevallier *et al.*, 2016; Joyce *et al.*, 2017). As previously discussed, such mechanisms can increase the particle size and viscosity of food systems, resulting in deviation from typical rheological behaviour.

The inverse temperature-viscosity relationship of liquid foods may be described by the Arrhenius equation, written in the form:

$$\mu_T = \mu_o \exp \frac{-E_a}{RT} \qquad 1.3$$

where μ_T (in Pa.s) is the apparent viscosity at temperature T (in K), μ_o (in Pa.s) is the asymptotic viscosity as T approaches infinity, E_a is the activation energy of the system (in kJ/mol) and R is the universal gas constant (8.314 J/mol/K). The Arrhenius equation describes the rate of a process which increases monotonically with temperature and asymptotically approaches a constant value and is one of the most widely used temperature-viscosity models (Recondo *et al.*, 2006; Peleg, 2017). In addition to its use in rheological modelling, the Arrhenius equation is applied to describe a wide range of chemical and biochemical reactions including modelling the kinetics of whey protein denaturation and microbial inactivation (Peleg, 2017).

Within the Arrhenius equation, E_a, denotes the energy that must be reached before the elementary flow process can occur. The Boltzmann factor $\left(\exp \frac{-E_a}{RT} \right)$ relates to the fraction of molecules which possess the required energy (Rao, 2013). Recently, the

suitability of the Arrhenius equations' widespread application in food systems has been debated. The equation is often applied empirically without reference to the fundamental physical basis on which it was developed. As many biological and chemical processes in food do not follow fixed-order kinetics, E_a is often ill-defined and unverified in food systems and the gas constants use in liquid food systems contribute to such concerns (Peleg, 2012; Saguy, 2016; Peleg, 2017). The use of alternative empirical models, without E_a or R terms, has been suggested as a more appropriate alternative to the Arrhenius equation in such cases (Peleg, 2017).

The Arrhenius equation has been applied in a wide range of ways to model the viscosity of milk systems (Alcântara *et al.*, 2012; Işıklı *et al.*, 2015). The Arrhenius equation was used to quantify changes in flow properties of concentrated milks during storage (Vélez-Ruiz and Barbosa-Cánovas, 1998). Gonçalves *et al.* (2017) utilised the Arrhenius equation to describe the rheological behaviour of commercial fermented dairy products including yogurts and dairy beverages, to aid in the optimisation of manufacture processes for the products. The equation has also been applied to dairy alternative beverages such as oat milk to quantify the effect of temperature and concentration on the rheology of the product (Deswarl *et al.*, 2014).

The Williams-Landel-Ferry (WLF) equation was developed for mechanical relaxation processes whose rate parameters, such as viscosity, compliance and stress, are related to the difference between the actual temperature of the material, T, and the glass transition temperature, T_g (Williams *et al.*, 1955; Ferry, 1980; Buera and Karel, 1993). The equation is expressed as:

$$ln\, a_T = \frac{-C_1(T-T_s)}{(C_2+T-T_s)} \qquad 1.4$$

where C_1 and C_2 are constants, and a_T is the ratio between the viscosity at temperature T and the viscosity at a selected (base) temperature, T_s, i.e.

$$a_T = \frac{\mu(T)}{\mu(T_S)} \quad 1.5$$

By combining equations 1.4 and 1.5, a general WLF equation for viscosity can be written as (Williams *et al.*, 1955; Peleg, 1992; Peleg, 2017).

$$ln\,\mu(T) = ln\,\mu(T_S) - \frac{C_1(T-T_S)}{(C_2+T-T_S)} \quad 1.6$$

In its original application, the glass transition temperature of the material, T_g was used as the selected temperature, T_s. However, while T_g can be measured by different techniques such as differential scanning calorimetry (DSC), viscosity measurements in the glass transition region are not achievable for all materials, e.g. the glass transition temperature of a liquid food might be far below the range of the model (Peleg *et al.*, 2002; Recondo *et al.*, 2006; Peleg, 2017). Another issue with T_g as a reference temperature, identified by Peleg, is that it is an "ill-defined temperature" which can often occur over a temperature range and whose value can vary as a result of the method used for its determination; therefore, other temperatures are often applied as T_s for the WLF equation (Peleg *et al.*, 2002); this amounts to using the WLF equation as an empirical fit to viscosity data without reference to glass transition. Prior to the development of the WLF equation, the same arbitrary reference temperature of 298 K or ~25 °C, was selected for each material in order to facilitate comparison between different systems. However, Williams *et al.* (1955) proposed that the reference temperature, T_s, be selected based on suitability for each system and that results be expressed as a function of $T\text{-}T_s$ to ease comparison between systems.

The WLF model is based on a phenomenon which occurs between T_g and T_g + 100°C for amorphous solids and super-cooled liquids, in which the temperature-dependence of viscosity is independent of molecular structure. As a result, the viscosity of a wide variety of these amorphous solid and super-cooled liquid materials behaves in a similar way and can be described by a "universal function". This universal function was developed using the composite curves for various polymers where $T_s = T_g + 50$ K, resulting in average values of 8.86 (-) and 101.6 (K) for C_1 and C_2, respectively. The values obtained for a_T for these composite curves and the application of the WLF equation were in agreement, except where $T < T_g$, as the equation cannot predict below the glass transition temperature, as it predicts the monotonic decrease of log a_T with increasing temperature instead of the inflection point, which is generally agreed to exist near T_g. These values were used to determine the universal equation, where $T_s = T_g$, written as:

$$log\ a_T = \frac{-17.44(T-T_g)}{(51.6+T-T_g)} \qquad 1.7$$

These values determined for C_1 and C_2 (i.e. C_1 = -17.44 and C_2 = 51.6) are often referred to as the "universal constants". However, the authors of the WLF model advise against the use of these "universal" coefficients (Williams *et al.*, 1955), and Ferry (1980) stated that these values can only be used as a last resort, when no reliable data are available from another source (Buera and Karel, 1993). A number of studies have applied the universal coefficients with limited success (Soesanto and Williams, 1981; Roos and Karel, 1990; Roos and Karel, 1991a; Roos and Karel, 1991b; Recondo *et al.*, 2006). The inaccuracy of these universal constants is due to their estimation based on synthetic polymer materials resulting in a limited application in food systems. The WLF equation is therefore most commonly applied in its general form,

with coefficients calculated for each material in order to avoid extrapolation (Ferry, 1980; Peleg, 1992; Recondo *et al.*, 2006).

From its origins in polymer science, the WLF equation has found relatively extensive application in food research and has been proposed as a replacement for the commonly used Arrhenius equation (Slade *et al.*, 1991; Sapru and Labuza, 1993; Sopade *et al.*, 2003; Peleg, 2017). This is due to the improved fits achieved by the WLF equation in place of the Arrhenius equation, in many cases, which may be attributed to the additional fitting parameter of the WLF equation. The WLF equation has been applied to a wide range of food systems, with particular focus on honey (Bhandari *et al.*, 1999; Mossel *et al.*, 2000; Sopade *et al.*, 2003; Juszczak and Fortuna, 2006; Recondo *et al.*, 2006; Ahmed *et al.*, 2007). In a dairy space, the equation has been applied to model non-enzymatic browning in non-fat milk and whey protein powders (Buera and Karel, 1993), estimate the activation energy of gelation of whey proteins (Katsuta and Kinsella, 1990), and model the viscoelasticity in, condensed whey protein systems (Dissanayake *et al.*, 2012), whey protein/lactose systems (Dissanayake, 2013), and crystallisation (Peleg, 1992; Roos and Karel, 1992) and stickiness in amorphous lactose (Roos and Karel, 1992; Paterson *et al.*, 2005).

The Power Law is an effective tool for determining the closeness of fluid to Newtonian behaviour, expressed as:

$$\sigma = K\dot{\gamma}^n \qquad 1.8$$

where K is the consistency coefficient (Pa.s) and is taken as the shear stress at a rate of 1 1/s and n is the behaviour flow index, a dimensionless number that reflects closeness to Newtonian flow. When n = 1, the fluid can be considered Newtonian, while fluids with n < 1 are shear-thinning and n > 1 are shear thickening. These

classifications indicate the type of response a fluid has under the application of shear (Fig. 1.8).

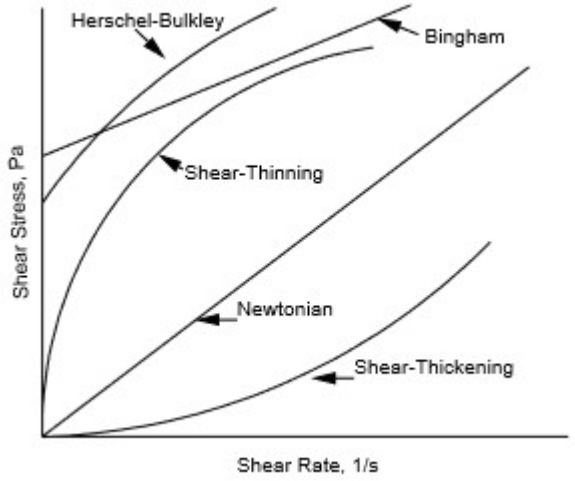

Fig. 1.8. Rheogram indicating typical shear rate – shear stress curves for fluids with different rheological behaviours (Steffe, 1996).

The work completed by Gonçalves *et al.* (2017) on commercial fermented dairy products encompassed analysis of rheological behaviour through the application of the Power Law, in addition to the Arrhenius equation. The equation has been used in conjunction with a wide range of dairy-based products such as lactose free dairy desserts (Sahin *et al.*, 2016), Dulce de leche (Rovedo *et al.*, 1991), yogurt (Shaker *et al.*, 2000), and oat starches containing milk ingredients (Kumar *et al.*, 2018).

Recently the Power Law has been applied to work in milk concentrates providing valuable information for processes like evaporation in which viscosity changes of high solids systems can have significant impact (Anema *et al.*, 2014; Kasinos *et al.*, 2015; Munir *et al.*, 2016; Sutariya *et al.*, 2017; Murphy *et al.*, 2018; Mercan *et al.*, 2018). The application of the Power Law demonstrated that increasing preheat temperature of non-Newtonian skim milk concentrates increased viscosity and the degree of shear-

thinning behaviour increased (Sutariya *et al.*, 2017). These effects were found to be correlated to increases in volume fraction and the extent of protein denaturation, rather than the effects of concentration. The effect of high pressure homogenisation (HPH) on skim and whole milk concentrates was evaluated using the Power Law which revealed that the n decreased indicating an increase in shear-thinning behaviour as the HPH pressure increased, 0 – 40 MPa and 0 – 150 MPa for whole and skim milks respectively (Mercan *et al.*, 2018). This work indicated that the rheological behaviour of milk concentrates could be significantly impacted by the application of HPH.

There is generally a direct nonlinear relationship between concentration and viscosity at a constant temperature (Bourne, 2002). This relationship can be described through viscosity models, considering viscosity as a function of the volume fraction. When milk is heat-treated, whey proteins denature and aggregate with themselves and casein micelles, increasing the voluminosity of the system and affecting viscosity. This change can be described using equations like the Einstein and Eilers equations which apply the theory of hard spheres to account for changes in volume fraction and interactions between casein micelles due to thermal processing (Jeurnink and De Kruif, 1993).

The Einstein equation was first developed in 1906 based on dilute rigid sphere dispersions (Einstein, 1906; Rao, 2013). It describes the relationship between viscosity, μ, and the volume fraction, ϕ, of dilute dispersions of hard spheres in a continuous phase of known viscosity, μ_s, where μ/μ_s can be taken as the relative viscosity, μ_r (Anema *et al.*, 2004; Rao, 2013).

$$\mu_r = \frac{\mu}{\mu_s} = 1 + 2.5\phi \qquad 1.9$$

This equation tends to be more suitable for dilute suspensions, with skim milk being considered a concentrated suspension. Dilution of skim milk may be required to calculate the volume fraction of casein micelles using this equation (Anema *et al.*, 2004; Anema *et al.*, 2014).

The viscosity of skim milk can also be described by the semi-empirical Eilers equation (Eilers, 1941; Walstra and Jenness, 1984):

$$\mu_r = \frac{\mu}{\mu_s} = \left(1 + \left(\frac{1.25\phi}{1-\phi/\phi_{max}}\right)\right)^2 \qquad 1.10$$

This equation has proved to be more suitable for concentrated systems like milk and milk concentrates (Dewan *et al.*, 1973; Anema *et al.*, 2014). This equation has been applied to investigate the viscosity of skim milk concentrate revealing a large dependence on alterations to pH and NaCl concentration (Karlsson *et al.*, 2005).

Both the Euler and Einstein equations have been applied in studies of reconstituted skim milk by Anema and Li (2003) and Anema *et al.* (2004 and 2014). The application of these equations found that changes in viscosity and casein micelle volume could be correlated to the extent of protein denaturation across skim milks with adjusted pH (Anema *et al.*, 2004).

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Objectives

The nutritional quality and functional properties of dairy protein beverages have been leveraged to build a rapidly growing, global nutritional and performance beverage market (Cochrane et al., 2012; Mintel, 2018). The high degree of thermal treatment required to produce ready-to-drink, shelf-stable variants of these beverages can negatively impact their physical, nutritional and sensorial characteristics (LaClair and Etzel, 2010; Brodkorb *et al.*, 2016; Wagoner and Foegeding, 2017). The objective of this research was to investigate beverage formulation and thermal processing techniques, assessing potential to minimise thermally-induced changes, across a range of model protein-enriched dairy beverages with different casein to whey protein (CN:WP) ratios which were reflective of the current market. This was achieved through investigating the effect of composition and protein profile on the thermal stability, in terms of viscosity, of protein-enriched dairy beverages during laboratory-scale heat treatment. A second study investigated the effect of protein profile on the physical characteristics of tubular heat-treated protein beverages. The impact of thermal processing technology on the physical characteristics of protein-enriched beverages was examined for whey protein- and skim milk-based beverages (0:100 and 80:20 CN:WP respectively) with different total protein contents. Three thermal processing technologies formed the basis of these studies; indirect tubular heating, direct steam infusion, and novel direct supersonic steam injection heating. These technologies were applied across a range of extended shelf life and ultra-high-temperature heat treatments. Comprehensive evaluation of these heat treatment technologies in the protein-enriched dairy beverage space underpins innovation in food manufacturing and expands the knowledge base to support consumer-focused product development.

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Chapter 2.

Evaluation of temperature-dependent models of viscosity changes in dairy protein beverage formulations during thermal processing

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Kelleher, C.M., O'Mahony, J.A., Kelly, A.L., O'Callaghan, D.J. and McCarthy, N.A. (2018). Evaluation of temperature-dependent models of viscosity changes in dairy protein beverage formulations during thermal processing. Journal of Food Science, 83, 937 – 945.

2.1. Abstract

Rheological modelling as a function of temperature is a useful tool for describing products undergoing thermal processing. The rheological behaviour of a range of dairy-based (4%, w/w) protein beverages was investigated for applicability of semi-empirical temperature-dependent viscosity equations. The viscosity at 16.8 rad/s of the beverages was measured during heating, holding and cooling over a temperature range of 25-90 °C using a rheometer with starch pasting cell geometry. Five established fitting methods were applied based on the Arrhenius and Williams-Landel-Ferry (WLF) equations using non-linear regression analysis. A two-parameter WLF (WLF_2) model, using viscosity at a reference temperature of 25 °C resulted in high R^2 values (0.974 – 0.988) and a statistically superior fit compared to the Arrhenius, Generalised Arrhenius and Exponential equations ($p < 0.001$). Deviation from the WLF_2 modelled equation was used to describe and investigate the effect formulation had on the changes in viscosity during thermal heating. This study successfully applied the WLF equation to a liquid protein system, proving that a consistent and close fit can be achieved across a range of formulations. A rapid, quantitative method for viscosity-temperature profile evaluation is presented, which can ease product development and optimisation of product processing stability.

2.2. Introduction

The use of whey protein ingredients in beverages for specialised uses is growing rapidly. These specialised beverages include nutritional products for the elderly, meal replacement drinks, low-sugar drinks for diabetic patients, and highly functional sports foods for high performance athletes and body-builders (Shiby, 2013). In addition to protein, which provides amino acids for muscle recovery and repair, these beverages often contain carbohydrates as a source of energy. Formulating such heat-stable protein-carbohydrate nutritional beverages can be challenging (Chen and O'Mahony, 2016).

Heat treatment is carried out on dairy beverages with the aim of reducing the microbial population, inactivating enzymes, and minimising chemical reactions and physical changes in the product during storage (Lewis and Deeth, 2009). During heat treatment, a number of thermally induced physical changes occur in dairy-based beverages. Whey proteins undergo conformational changes during heating, due to unfolding of their native compact globular structures (i.e. protein denaturation and aggregation) which result in technical challenges that may negatively impact process efficiency and product quality (Wijayanti *et al.*, 2014; Joyce *et al.*, 2017). These denaturation and aggregation mechanisms, at temperatures greater than 75ºC, can lead to fouling of heat-exchangers, increased turbidity, sedimentation and viscosity of beverages with a protein concentration greater than 3.5%, w/w (Joyce *et al.*, 2017). Fouling is a major processing issue in the dairy industry, where up to 80% of operational costs can be related to fouling, shutdown and cleaning processes (De Jong, 2008). Attempts to reduce fouling within thermal processing include increasing product heat stability, reducing temperature and residence time, increasing flow velocities and turbulence, and monitoring pH (Feldman, 2016; Santos *et al.*, 2006).

Rheological characterisation is used in process engineering, quality control and product development (Messaâdi *et al.*, 2015), and changes in rheological properties, such as viscosity, have been long-established as indicators of protein denaturation, aggregation and fouling (Wallhäußer *et al.*, 2012). Heat stability can also be determined from viscosity measurements at high temperatures, as the onset of coagulation can be detected by rheological analysis (Huppertz, 2016). Thus, characterising the rheological behaviour of a dairy protein beverage under a defined heating cycle can provide useful insights into its behaviour during thermal processing, aiding formulation design. Mathematical modelling of viscosity can be employed to reduce a large quantity of rheological data to mathematical equations that can be related to physical changes, easing the description of this rheological behaviour (Steffe, 1996; Saguy, 2016).

A number of temperature-dependent viscosity models have been cited, the most prevalent of which are forms of Arrhenius and Williams-Landel-Ferry (WLF) models. The Arrhenius equation describes the rate of a process which increases monotonically with temperature and asymptotically approaches a constant value and is commonly used to describe the effect of temperature on kinetics of chemical and biochemical reactions. It has been used extensively to describe the temperature-dependence of viscosity in both Newtonian and non-Newtonian materials (Recondo *et al.*, 2006). The Arrhenius equation has been used in dairy applications, e.g., to model the kinetics of whey protein denaturation (Jeurnink *et al.*, 1996a; Wolz and Kulozik, 2015; Blanpain-Avet *et al.*, 2016; Brodkorb *et al.*, 2016) and microbial inactivation (Lobacz and Kowalik, 2015; Kim *et al.*, 2016), while other studies have applied this equation in examining the effect of temperature on dynamic viscosity (Alcântara *et al.*, 2012) and the effect of viscosity on the flow behaviour of flavoured milk drinks (Işıklı *et al.*,

2015). However, concerns have been raised about its widespread application, as the equation is often applied empirically rather than as a fundamental physical model, which can lead to issues in its application to some chemical and biological processes in food which do not completely follow first- or fixed-order kinetics (Saguy, 2016). Peleg (2012) suggested that the Arrhenius equation is unsuitable for food systems as the activation energy (E_a) determined is ill-defined and unverified, and the gas constant (R) holds no bearing over a food system. It has therefore been suggested that a simplified empirical model, without E_a or R terms, such as the Generalised Arrhenius and Exponential equation may be more appropriate (Peleg *et al.*, 2017).

The WLF equation has also been proposed as an alternative for describing the temperature dependence of food systems in place of the Arrhenius equation in food literature (Slade *et al.*, 1991; Sapru and Labuza, 1993; Sopade *et al.*, 2003), as the two adjustable parameters can provide a better fit than the single Arrhenius parameter (Peleg, 2012). The WLF equation was used initially to relate viscosity to temperature in amorphous materials, using glass transition temperature (T_g) as a reference (Williams *et al.*, 1955; Ferry, 1980). While originally used to study the viscosity of polymers, the WLF equation has been applied to food systems, such as honey (Mossel *et al.*, 2000; Sopade *et al.*, 2003; Ahmed *et al.*, 2007) and a variety of dairy applications, such as modelling non-enzymatic browning in non-fat milk and whey protein powders (Buera and Karel, 1993). It also has been used to estimate the activation energy during whey protein gelation (Katsuta and Kinsella, 1990), and to model viscoelasticity in whey protein/lactose systems (Dissanayake, 2013), and crystallisation (Peleg, 1992; Roos and Karel, 1992) and stickiness in amorphous lactose (Roos and Karel, 1992; Paterson *et al.*, 2005). While the application to beverage systems in the current study falls outside the originally specified use with

amorphous and super-cooled liquids, the model is applied in an empirical fashion, in a similar manner to applications cited for other dilute food systems (Rao, 2013).

The rheological behaviour of foods, such as protein-rich beverages, is complex and influenced by numerous mechanisms and factors, but the fitting of appropriate mathematical equations could make the comparison between product composition and the effects of heat treatment easier (Steffe, 1996). The aim of this study was to investigate the application of the WLF-based equations to the viscosity-temperature profiles of dairy protein formulations, comparing then against the well-established and modified versions of the Arrhenius equation, and to investigate the application of an appropriate viscosity model to reduce rheological data to a single value as a means of accelerating formulation development of heat-treated protein-rich beverages.

2.3. Materials and methods

2.3.1. *Materials*

Whey protein isolate (BiPro®) (composition: 91.8%, w/w, protein, 0.2%, w/w, fat, 2.0%, w/w, ash and <0.2%, w/w, lactose) was supplied by Davisco Foods International (Le Sueur, MN, USA). Skim milk powder (SMP) (composition: 39.9%, w/w, protein, 0.9%, w/w, fat, 46.6%, w/w, lactose and 7.9%, w/w, ash) was supplied by Tipperary Co-operative (Tipperary Town, Co. Tipperary, Ireland). Lactose was supplied by Glanbia Ingredients Ireland Ltd. (Ballyragget, Co. Kilkenny, Ireland). Maltodextrin (moisture and ash levels of <5.0%, w/w, and <0.5%, w/w, respectively, and a dextrose equivalent value of 16) was supplied by Cargill CTS (Haubourdin, France).

2.3.2. *Model beverage formulation*

Five formulations were produced with two experimental design variables: protein source, i.e., whey protein or a 60:40 blend of whey protein and casein, and

carbohydrate content, i.e., varying lactose and maltodextrin levels (Table 2.1). The total protein content of each formulation was 4%, w/w. Distilled water was heated to 42ºC to aid reconstitution of the powder ingredients, which were subsequently added and mixed using a magnetic stirrer at ~200 rpm for 2 h. The pH of the formulation was adjusted to 6.8 using < 500 μL of sodium hydroxide and/or hydrochloric acid solutions at 0.1 M, if required. After mixing, the samples were stirred gently overnight at 4ºC to ensure complete hydration. Experimental batches were prepared separately, in triplicate.

Table 2.1. Dairy protein beverage formulation composition (g/100 g) [1]

Formulation[2]	W	WL	WCL	WLM	WCLM
Whey protein	4.00	4.00	2.40	4.00	2.40
Casein	0.00	0.00	1.60	0.00	1.60
Lactose	0.00	2.35	2.35	2.35	2.35
Maltodextrin	0.00	0.00	0.00	2.35	2.35
Total solids	4.35	6.70	7.16	9.05	9.51

[1] The composition is calculated from the amount of each ingredient added, based on known ingredient composition, given in Section 2.1

2 The formulations are labelled with regard to their composition: whey protein only (W), whey protein and lactose (WL), whey protein/casein and lactose (WCL), whey protein and lactose/maltodextrin (WLM), and whey protein/casein and lactose/maltodextrin (WCLM).

2.3.3. *Rheological analysis*

The rheological behaviour of the formulations under shearing conditions was determined using an AR 2000ex rheometer (TA Instruments, Crawley, UK) with a

concentric cylinder geometry. A shear rate sweep was completed for all five formulations from 0 – 200 1/s at 25ºC and the Power Law was applied to the shear rate versus shear stress measurements:

$$log\,\sigma = log\,K + n\,log\,\gamma \quad 2.1$$

The value of n, a dimensionless number, indicates a fluids closeness to Newtonian flow at a value of 1. The rheological behaviour of all five formulations under shear conditions was determined using the Power Law, resulting in n values between 1.03 and 1.06. As a result, the formulations can be considered relatively Newtonian under various shear rate conditions at a constant temperature, therefore, validating the use of viscosity data at the single chosen angular velocity.

Apparent viscosity as a function of temperature was measured using the AR 2000ex rheometer paired with a starch pasting cell geometry. Temperature was controlled by peltier heating and air-water coolant circulation, as required. A temperature sweep was performed at a constant angular velocity of 16.8 rad/s over a heating step from 25 ºC to 90 ºC at 2.5 ºC/min, a holding step at 90 ºC for 5 min, and a cooling step to 25 ºC at 2.5 ºC/min. Previous work carried out by Feldman (2016) showed that the Microthermics tubular heat exchanger system operates with transitional flow at a flow rate of 3 L/min, as does the starch pasting cell when rotating at an angular velocity of 16.8 rad/s, based on Reynolds number calculations. The transitional flow behaviour was validated using Reynolds number, between Re 2000 and 4100. Similar flow profiles between the two methods allow for the simulation of pilot-scale processing conditions at lab scale and are useful in measuring viscosity of protein formulations (Murphy *et al.*, 2014; Joyce *et al.*, 2017).

2.3.4. *Curve fitting to temperature-dependent viscosity models*

Established models were fitted to viscosity data for each upward and downward temperature sweep using the generalised reduced gradient (GRG Nonlinear) algorithm in the Solver add-in of Microsoft Excel 2010 (Lasdon *et al.*, 1974). The model parameters were determined by least squares regression, minimising the sum of squared residuals (SSR):

$$SSR = \sum_{j=1}^{n_p} \left(\mu_j - \mu\right)^2 \qquad 2.2$$

where n_p is the number of data points in the temperature ramp being fitted, μj is the viscosity (Pa.s) measured at the j^{th} instant of time, and μ is the corresponding model prediction of viscosity (Tibäck *et al.*, 2014).

2.3.4.1. Arrhenius-based models

The Arrhenius equation, applied to viscosity, can be expressed in its simplest form as:

$$ln\mu(T) = ln\mu_o + \frac{E_a}{RT} \qquad 2.3$$

where μ is viscosity (Pa.s), μ_o is an asymptotic viscosity as T approaches infinity, E_a is the activation energy for the reaction (kJ/mol), R is the gas constant (8.314 J/mol/K) and T is the absolute temperature (K).

A Generalised Arrhenius equation has been proposed by Peleg (2017):

$$ln\mu(T) = ln\mu_o + a\left(\frac{1}{T} - \frac{1}{T_o}\right) \qquad 2.4$$

where T and T_o are in K and the constant a replacing the E_a/R term in the standard Arrhenius equation, has units of temperature. Peleg (2017) argues against the use of an explicit E_a/R term in complex food systems.

Peleg applied the Saravacos Exponential equation, in log-transformed form:

$$ln\mu(T) = A - BT \quad 2.5$$

where A and B are constants and T is in °C (Saravacos, 1977; Peleg, 2017).

The Arrhenius, Generalised Arrhenius equations express linear behaviour in $ln\mu(T)$ versus 1/T plots, often described as Arrhenius-type behaviour (Messaâdi *et al.*, 2015). The Generalised Arrhenius and Exponential equations are presented here as alternatives to the simple Arrhenius equation, replacing the E_a and R terms with alternative constants and thereby removing the issues related to the physical meaning of a "mole" in liquid food systems (Saguy, 2016; Peleg, 2017).

2.3.4.2. Williams Landel Ferry model

The Williams Landel Ferry (WLF) equation produced by Williams *et al.* (1955) was expressed as:

$$ln\mu(T) = ln\mu(T_s) - \frac{C_1(T-T_s)}{(C_2+T-T_s)} \quad 2.6$$

where C_1(-) and C_2 (K) are constants, and T_s is a selected reference temperature (K).

In this study, the WLF equation was applied in two ways, namely a WLF four-parameter (WLF_4) and a WLF two-parameter (WLF_2) approach. The WLF_2, as recommended by Peleg (1992), uses a reference temperature (T_s) of 25°C, where $\mu(T_s)$ is the measured viscosity at 25°C, giving two fitting parameters, C1 and C2, fitted

using non-linear least squares regression. The WLF4 method optimises all four parameters in Equation 7 using this regression technique: C1, C2, T_o and μ_o.

$$ln\mu(T) = ln\mu_o - \frac{C_1(T-T_o)}{(C_2+T-T_o)} \quad 2.7$$

Parameters C_1 and C_2 are determined through non-linear regression in both the WLF_4 and WLF_2 applications.

2.3.5. *Statistical analysis*

R^2 values were used to comparing the fits of different viscosity models. Steiger's Z test statistic was used to determine if correlations were overlapping or different and identify statistical significance of R^2 results (Steiger, 1980). All calculations were carried out using Microsoft Excel 2010. One-way ANOVA was applied to evaluate statistical differences in determined coefficients and R^2 values for formulations using the MINITAB® 15 (Minitab Ltd., Coventry, UK) statistical analysis package.

2.4. Results and discussion

2.4.1. *Viscosity-temperature profiles*

Viscosity-temperature profiles exhibited similar overall trends for all five formulations; viscosity generally decreased as temperature increased, and subsequently increased as temperature decreased (Fig. 2.1 and Table 2.2). However, at the higher temperatures of the upward temperature ramp, deviation from this behaviour was observed, i.e., at approximately 70°C and above, the decline in viscosity slowed and viscosity began to increase, even as temperature increased. This is likely the result of whey protein denaturation and aggregation contributing to increased viscosity of the formulations, as these mechanisms are known to occur at

temperatures exceeding 70°C resulting in changes in viscosity (Chevallier et al., 2016; Joyce et al., 2017). Fitzsimons et al. (2007), using a similar composition to that used in this study, found by differential scanning calorimetry (DSC) analysis that, at 3.0% w/w whey protein, denaturation of β-lactoglobulin (β-lg) and α-lactalbumin (α-la) was initiated at ~75°C and ~62°C, respectively. Joyce et al. (2017) reported extensive protein denaturation, in a study of model infant nutritional product formulations (5.2% w/w protein with a whey protein: casein ratio of 60:40 formulated using the same commercial WPI and low-heat SMP) heated at 85°C for 2 min, with a total protein denaturation level of 81.2% and β-lg denaturation of 94.7%.

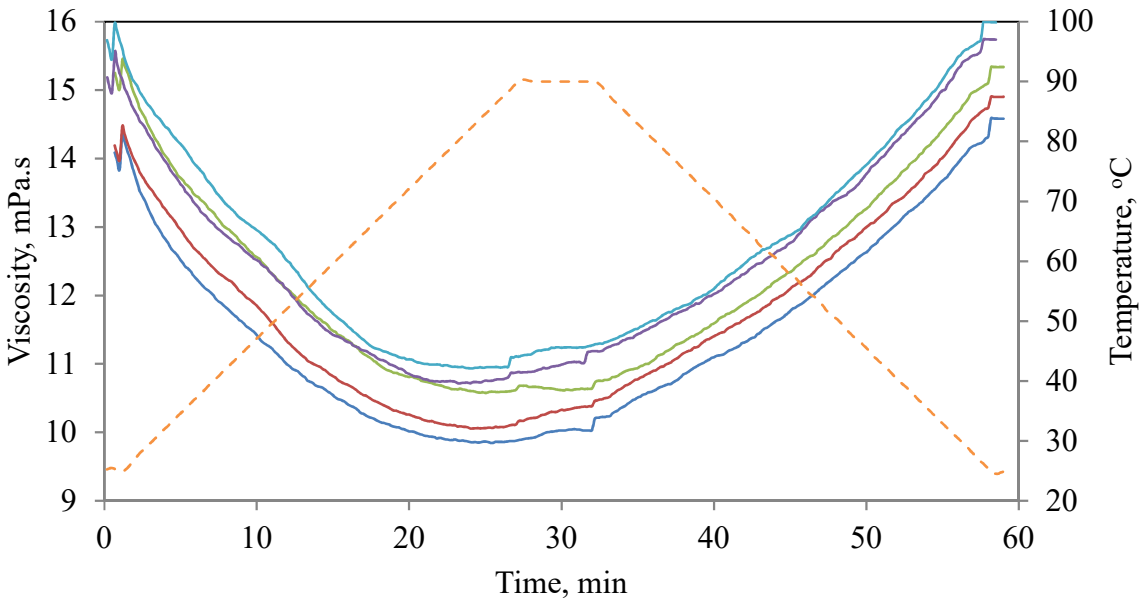

Fig. 2.1. Viscosity profiles during heating from 25 to 90 °C, holding at 90 °C for 5 min and cooling from 90 to 25 °C and temperature profile (——) for whey protein beverage formulations W (—), WL (—), WCL (—), WLM (—), WCLM (—), illustrating the slowed decrease in viscosity and onset of increasing viscosity from ~70 °C during the upward temperature ramp.

Table 2.2. The slope of temperature segments of the upward, holding and downward temperature ramps of the beverage formulations viscosity profile in mPa.s/min

Formulation	Increasing temperature		Hold	Decreasing temperature	
	25-70 °C	70-90 °C	90 °C	90-70 °C	70-25 °C
W	-0.22	-0.03	0.03	0.12	0.19
WL	-0.22	-0.03	0.04	0.13	0.19
WCL	-0.24	-0.04	0.01	0.11	0.20
WLM	-0.24	-0.03	0.04	0.11	0.21
WCLM	-0.26	-0.02	0.02	0.10	0.21

In our study, the downward temperature ramp did not display the same changes in rheological behaviour as the upward temperature ramp, and viscosity increased continuously as temperature decreased through the range 90-25°C. In general, the viscosity of formulations increased with increasing total solids content (Table 2.1) as reported by others (Patocka *et al.*, 2006).

2.4.2. *Arrhenius-based models*

The parameters determined for the Arrhenius-based equations (Arrhenius, Generalised Arrhenius and Exponential equations) investigated did not differ to a significant degree between the beverage formulations ($p > 0.05$), with the exception of the A term of the Exponential equation (Table 2.3). The A values determined decreased with increasing total solids concentration of the beverage formulations, and statistical differences were observed between the parameters determined for formulations containing maltodextrin (i.e. WLM and WCLM) and those without ($p < 0.05$).

The downward temperature ramp was well described in all cases by the Arrhenius equation (R^2, 0.981 - 0.986) and its derivatives, the Generalised Arrhenius equation (R^2, 0.981 – 0.986) and the Exponential equation (R^2, 0.971 – 0.979). However, a relatively poorer fit was obtained when the equations were fitted to the upward temperature ramp, with R^2 fits ranging from 0.933 – 0.947 for the Arrhenius and Generalised Arrhenius equations, and 0.908 – 0.924 for the Exponential equation (Fig. 2.2 and Table 2.4). For both upward and downward temperature ramps, the Arrhenius equation provided a significantly superior fit to the Exponential equation ($p < 0.001$). The R^2 values obtained for the Arrhenius equations were greater than those for the Generalised Arrhenius equation for all formulations ($p < 0.001$), with the exception of W on the upward temperature ramp where an equal fit was obtained using both equations ($p > 0.05$).

Table 2.3. Parameters determined for temperature-dependent viscosity models fitted to the viscosity data of beverage formulations W, WL, WCL, WLM, and WCLM during the (a) upward and (b) downward temperature ramps.

(A)	Arrhenius		Generalized Arrhenius			Exponential		WLF_4				WLF_2	
Form.	μ_o ln(Pa.s)	E_a kJ/mol	μ_o ln(Pa·s)	T_o K	a –	A –	B –	μ_o ln(Pa·s)	T_o K	C_1 –	C_2 K	C_1 –	C_2 K
W	1.65 ± 0.16	5215 ± 277	18.3 ± 0.48	259 ± 0.25	620 ± 27.3	−4.47 ± 0.01	5.69E-03 ± 2.53E-04	15.6 ± 0.28	289± 5.99	4.87 ± 0.04	4.49 ± 0.93	0.61 ± 0.01	36.7 ± 5.51
WL	1.68 ± 0.34	5253 ± 547	19.0 ± 1.02	260 ± 0.50	632 ± 65.8	−4.44 ± 0.01	5.83E-03 ± 6.47E-04	15.4 ± 0.45	289 ± 4.05	4.94 ± 0.04	7.08 ± 1.05	0.70 ± 0.07	52.8 ± 4.04
WCL	1.65 ± 0.16	5427 ± 323	20.4 ± 1.01	260 ± 0.35	653 ± 38.9	−4.38 ± 0.02	6.03E-03 ± 4.28E-04	15.5 ± 0.73	289 ± 23.5	4.96 ± 0.20	9.89 ± 7.43	0.77 ± 0.20	59.3 ± 29.1
WLM	1.82 ± 0.49	5246 ± 815	20.2 ± 1.79	260 ± 0.79	631 ± 98.1	−4.09 ± 0.07	5.81E-03 ± 9.14E-04	13.5 ± 3.10	287 ± 11.5	4.87 ± 0.15	7.03 ± 4.00	0.68 ± 0.17	49.2 ± 9.63
WCLM	1.66 ± 0.40	5554 ± 706	21.3 ± 1.77	260 ± 0.63	669 ± 85.0	−4.04 ± 0.07	6.17E-03 ± 7.98E-04	13.6 ± 2.98	287 ± 11.2	4.95 ± 0.15	10.1 ± 4.52	0.82 ± 0.22	65.1 ± 21.0
(B)	Arrhenius		Generalized Arrhenius			Exponential		WLF_4				WLF_2	
Form.	μ_o ln(Pa·s)	E_a kJ/mol	μ_o ln(Pa·s)	T_o K	a –	A –	B –	μ_o ln(Pa·s)	T_o K	C_1 –	C_2 K	C_1 –	C_2 K
W	2.00 ± 0.00	4903 ± 26.1	19.5 ± 0.29	293 ± 0.18	590 ± 3.14	−4.38 ± 0.01	5.45E-03 ± 3.12E-05	20.2 ± 1.55	293 ± 5.63	5.47 ± 0.04	35.6 ± 3.25	1.22 ± 0.09	158 ± 15.5
WL	2.07 ± 0.07	4872 ± 84.7	19.8 ± 0.30	293 ± 0.17	587 ± 10.2	−4.36 ± 0.01	5.41E-03 ± 1.01E-04	19.9 ± 1.75	293± 30.1	5.52 ± 0.29	41.7 ± 19.3	1.36 ± 0.49	184 ± 86.4
WCL	2.01 ± 0.09	5007 ± 177	20.5 ± 0.73	294 ± 0.35	603 ± 21.3	−4.15 ± 0.17	5.56E-03 ± 2.02E-04	16.5 ± 6.78	290 ± 21.5	5.40 ± 0.20	34.2 ± 12.1	1.19 ± 0.20	146 ± 27.9
WLM	2.17 ± 0.13	4887 ± 181	20.9 ± 0.56	294 ± 0.32	588 ± 21.8	−4.04 ± 0.02	5.42E-03 ± 2.08E-04	15.6 ± 5.85	289 ± 19.8	5.23 ± 0.18	27.1 ± 9.49	1.14 ± 0.17	145 ± 26.0
WCLM	2.20 ± 0.12	4875 ± 185	21.0 ± 0.70	294 ± 0.31	587 ± 22.2	−4.03 ± 0.03	5.40E-03 ± 2.15E-04	14.9 ± 5.37	288 ± 28.1	5.17 ± 0.23	24.4 ± 11.6	1.06 ± 0.26	130 ± 43.0

2.4.3. *Williams-Landel-Ferry Equation*

Each of the parameters identified for the WLF_4 and WLF_2 equations were statistically similar across formulations ($p > 0.05$; Table 2.3). A linear relationship was found between C_1 and C_2 for all formulations that was characteristic of upward and downward temperature ramps; a different relationship was found for WLF_4 and WLF_2 models. It can be inferred from Equation 6 that the curvature of a plot of log viscosity versus 1/T is related to C_2 and that curvature becomes insignificant, i.e., tends toward a straight line, when C_2 is much greater than the maximum value of $T - T_s$. The coefficient C_1 is influenced by C_2 and the overall slope of the plot. This effect can be seen in all formulation models, where lower C_2 values are reflected in greater curvature for the upward temperature ramp (Fig. 2.3 and Table 2.3). The fits obtained using the WLF equations, WLF_4 and WLF_2, were far superior to those using Arrhenius-based equations in all cases on upward and downward temperature ramps ($p < 0.001$; Table 2.4). This was particularly evident for the upward temperature ramp, where the average R^2 values of both WLF equations was 0.978 compared to an average of 0.933 for the three Arrhenius-based equations (Fig. 2.2). The downward temperature ramp was well described by most models, with the average R^2 value exceeding 0.983, with the exception of the Exponential equation (R^2 of 0.975); however, the application of the WLF equations provided the best fit (R^2 of 0.986).

Both the WLF_4 and WLF_2 equations provided statistically similar fits for the downward temperature ramp of all formulations ($p > 0.001$) (Fig. 2.2 and 2.4). On the upward temperature ramp, the WLF_4 equation gave a fit that was as good as, or in the case of the W and WLM formulations, better than that of the WLF_2 equation ($p < 0.001$). The WLF_2 equation allows for comparison of viscosity-temperature relationships for formulations using only two calculated parameters and maintains the

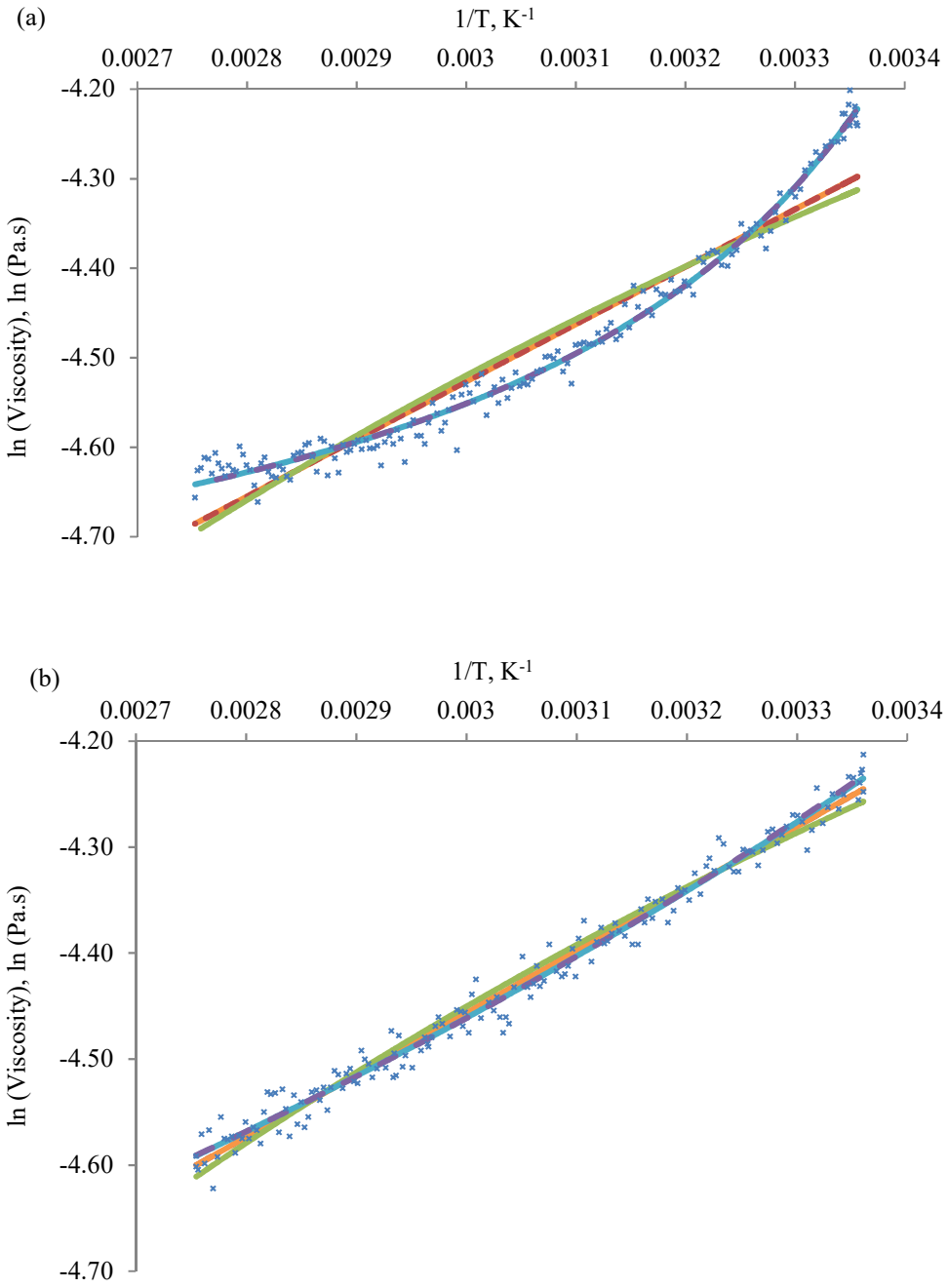

Fig. 2.2. Viscosity data (x), Arrhenius (---), Generalised Arrhenius (—), Exponential (—), WLF_4 (—), and WLF_2 (---) parameter models for the (a) upward and (b) downward temperature ramp for formulation W plotted on a logarithmic scale.

Table 2.4. R^2 statistical analysis of temperature-dependent viscosity models fitted to the viscosity data of beverage formulations W, WL, WCL, WLM, and WCLM during the (a) upward and (b) downward temperature ramps.

(a) Form.	Arrhenius	Generalised Arrhenius	Exponential	WLF_4	WLF_2
W	0.93 ± 0.03	0.94 ± 0.02	0.91 ± 0.03	0.98 ± 0.00	0.98 ± 0.00
WL	0.94 ± 0.02	0.94 ± 0.02	0.92 ± 0.02	0.98 ± 0.01	0.98 ± 0.01
WCL	0.95 ± 0.01	0.95 ± 0.01	0.92 ± 0.01	0.98 ± 0.01	0.98 ± 0.01
WLM	0.93 ± 0.02	0.93 ± 0.02	0.91 ± 0.02	0.98 ± 0.01	0.97 ± 0.00
WCLM	0.95 ± 0.00	0.95 ± 0.00	0.92 ± 0.01	0.97 ± 0.01	0.97 ± 0.01

(b) Form.	Arrhenius	Generalised Arrhenius	Exponential	WLF_4	WLF_2
W	0.99 ± 0.00	0.99 ± 0.00	0.98 ± 0.00	0.99 ± 0.00	0.99 ± 0.00
WL	0.98 ± 0.00	0.98 ± 0.00	0.98 ± 0.00	0.98 ± 0.00	0.98 ± 0.00
WCL	0.99 ± 0.00	0.99 ± 0.00	0.98 ± 0.01	0.99 ± 0.00	0.99 ± 0.00
WLM	0.98 ± 0.00	0.98 ± 0.00	0.97 ± 0.01	0.99 ± 0.00	0.99 ± 0.00
WCLM	0.98 ± 0.01	0.98 ± 0.01	0.97 ± 0.01	0.99 ± 0.00	0.99 ± 0.00

relationship between T_r and μ_r, unlike the WLF_4 equation for which these values are determined by regression. As the fit provided by the WLF_4 and WLF_2 equations is similar in most cases, the more parsimonious WLF_2 equation is preferred over the other equations applied in this study. Despite its original development for amorphous polymers, the WLF equation was successfully applied empirically to liquid dairy-protein formulations, resulting in a better fit than the traditionally applied Arrhenius-based equations.

2.4.4. *Application of the WLF_2 equation to the difference in viscosity on heating and cooling and its use in comparing formulations*

From the application of the various models to the range of formulations in this study, it could be seen that the cooling ramps of all formulations exhibited an inverse viscosity-temperature relationship (Fig. 2.3 and Table 2.4). The deviation of viscosity during the heating ramp from the Arrhenius-type behaviour exhibited by the cooling ramp can be attributed to protein denaturation and aggregation during the heating step.

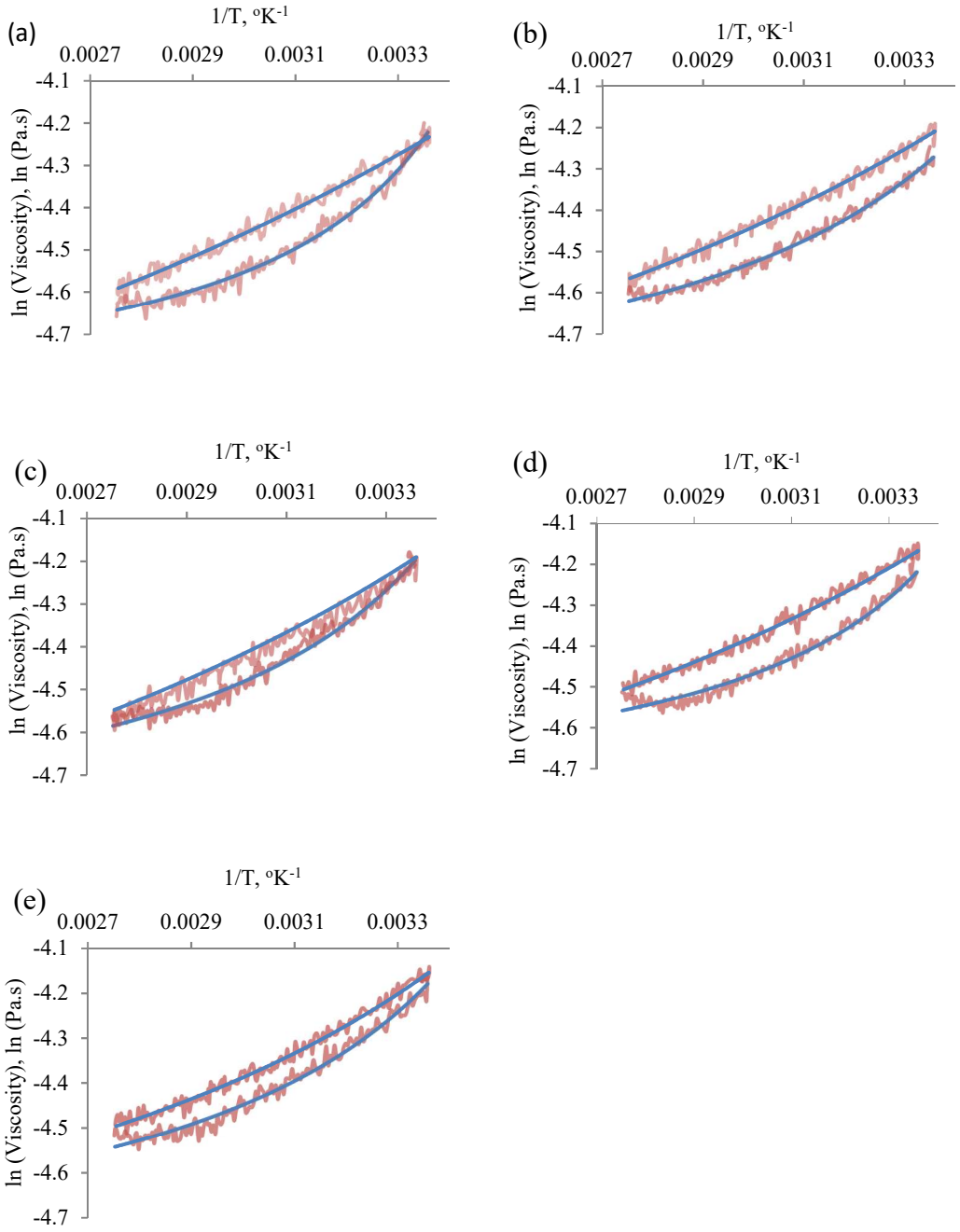

Fig. 2.3. WLF_2 equation (—) applied to viscosity on a logarithmic scale over upward and downward temperature ramps (—) for the beverage formulations, (a) W, (b) WL, (c) WCL, (d) WLM and (e) WCLM.

Thus, the deviation of the heating temperature ramp from the WLF_2-fitted cooling temperature ramp can be taken as a relative measure of the contribution of denaturation/aggregation to viscosity for the respective formulations (Fig. 2.4). The selected WLF_2 model was applied to the rheological data of each beverage formulation

and by using integration (area under the deviation with respect to time curve in log units to base 10 x seconds), the extent of this influence was analysed across the heating ramp, from 25ºC to 90ºC (i.e. 2 to 27 min).

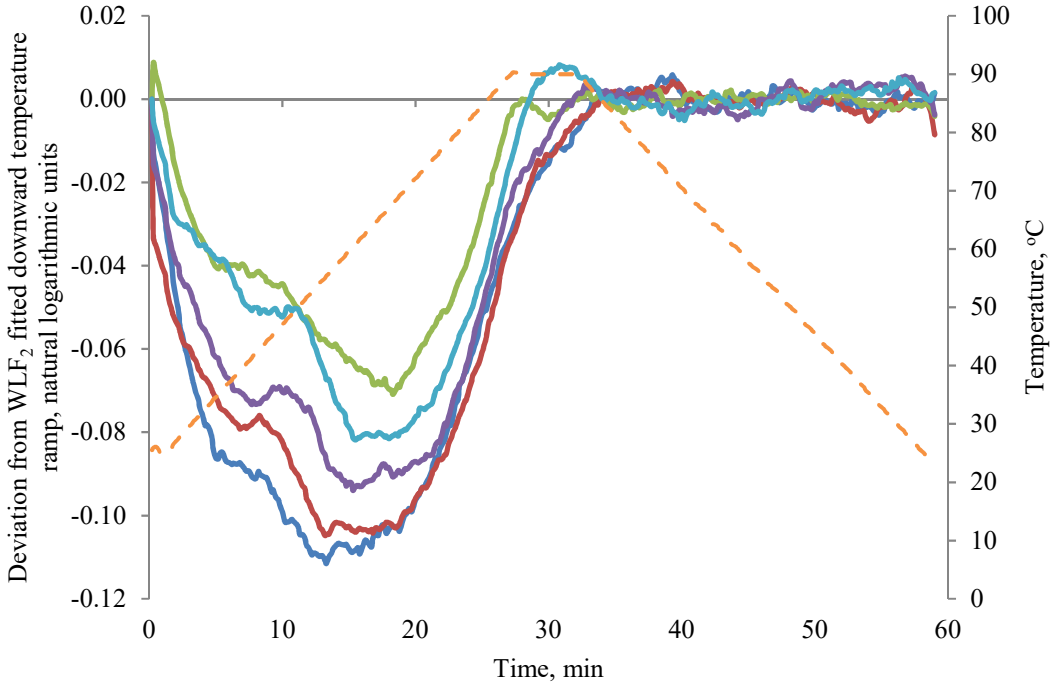

Fig. 2.4. Deviation in viscosity from the downward temperature ramp WLF_2 modelled across the heating cycle for formulations W (—), WL (—), WCL (—), WLM (—), and WCLM (—), illustrating the effect of temperature (---) on the viscosity of the formulations during the upward temperature ramp. Deviation was calculated as $(\ln(\mu_j)-\ln(\mu(T)))$, where μ_j is the viscosity at the j^{th} instant of time, and $\mu(T)$ is the viscosity at the same temperature (T) according to WLF_2 model.

Formulations with whey protein as the only protein source (W, WL, WLM) exhibited a greater overall increase in viscosity from heating ramp to cooling ramp (55.8, 52.8 and 45.4 log seconds, respectively) than formulations containing both whey protein and casein (WCL and WCLM at 29.7 and 35.3 log seconds, respectively) despite the latter formulations having higher solids contents (Fig. 2.4). The presence of casein and lactose in milk-based solutions has been shown to alter reaction rates and orders for whey protein denaturation, which in turn affects viscosity (Brodkorb *et al.*, 2016).

Previous studies have shown chaperone–like functions of casein, whereby the addition of casein to a whey protein system can facilitate improved heat stability and limit irreversible aggregation of whey proteins induced by thermal processing (O'Kennedy and Mounsey, 2006; Gaspard *et al.*, 2017). The same effect was seen by Chevalier *et al.* (2016), who reported that 4% protein solutions containing both whey protein and casein had increased heat stability after heat-induced aggregation of whey proteins.

For formulations containing whey protein as the sole source of protein, formulations W and WL had relative viscosity increases (integrated deviations of Fig. 2.4 plots) of 55.8 and 52.8 log seconds, respectively, while the whey protein-based formulation containing maltodextrin (WLM) showed a much lower viscosity increase, of 45.4 log seconds. The addition of maltodextrin to formulation WLM resulted in less overall viscosity deviation than for W and WL. Thus, in this study, the addition of lactose had a negligible effect on viscosity, while the impact of maltodextrin was much greater. Previous studies have found that whey protein denaturation mechanisms can be retarded in the presence of sugars such as lactose (Brodkorb *et al.*, 2016) and that increases in viscosity due to thermal processing can also be reduced by the presence of carbohydrates. Our findings are consistent with those reported by Crowley *et al.* (2014), who found that the addition of lactose to a dairy system can impair heat stability due to heat-induced acidification of the system, while maltodextrin has less impact on heat stability due to it having less reactivity during thermal processing. Similarly, Mulcahy *et al.* (2016) found that the addition of maltodextrin can improve heat stability of WPI dispersions containing 5% (w/w) protein and 5% (w/w) maltodextrin, which had similar overall composition to the formulations investigated in the current study. For formulations containing both whey protein and casein, namely WCL and WCLM, the viscosity deviations (at 29.7 and 35.3 log seconds,

respectively), were lower than for the whey protein-only formulations referred to above (55.8, 52.8 and 45.4 log seconds); however, the addition of maltodextrin (comparing WCL and WCLM) increased, rather than reduced, the viscosity deviation (Fig. 2.4). The approach of using a viscosity model to measure the impact of heat treatment, as outlined here, is a novel approach to quantitatively evaluating the effect of thermal processing on the viscosity of dairy formulations.

2.5. Conclusion

This study compared the novel empirical application of the WLF equation with the well-established Arrhenius equation for liquid dairy systems. The study statistically validated the use of the WLF equation for use with dairy products and showed that the WLF equation, applied empirically, can provide a better fit than the traditionally used Arrhenius equation. The Arrhenius, Generalised Arrhenius and Exponential equations proved to be inadequate for describing the temperature-dependence of viscosity in a system where heat-induced protein denaturation and aggregation occurs. The condensing of temperature-related viscosity data using the WLF equation allows for the prediction of thermal-induced viscosity issues. This model allows processors (i.e., dairy beverage and infant formula producers) to make informed assessments on the thermal stability of their products in terms of viscosity. Future work could also be extended to model the effects of minerals, pH and protein concentration on the viscosity behaviour of dairy formulations during thermal processing.

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Chapter 3.

The effect of protein profile and preheating on whey protein denaturation and viscosity of milk protein beverages during heat treatment

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3.1. Abstract

The effect of preheat temperature and casein to whey protein ratio (CN:WP) on the physical characteristics of 3.3%, w/w, dairy protein solutions was investigated. The choice of preheat temperature, 63 or 77 °C, with a final heat of 120 °C for 30 s resulted in reducing aggregate formation in the final protein solution of 77 °C-treated CN:WP of 0:100, compared to the lower preheat temperature and reduced viscosity for 77 °C-treated solutions reconstituted at a higher solids content. The CN:WP ratio significantly affected the properties of the heat-treated protein solutions. Casein-containing solutions (CN:WP ratios of 20:80, 50:50 and 80:20) had significantly lower levels of denaturation of α-lactalbumin. An increasing proportion of casein reduced changes in particle size due to heat treatment, with the 80:20 solution having no significant change in particle size on heating. However, the presence of casein also resulted in increased b* colour values, related to Maillard browning reactions, which can be perceived negatively by the consumer. The physical characteristics of a dairy protein system can be significantly influenced by altering the CN:WP ratio, with a higher proportion of casein improving thermal stability.

3.2. Introduction

Commercial production of milk, and its derivatives, requires effective heat treatment to ensure it is rendered microbiologically safe and physically stable for consumers. Therefore, the heat stability of milk and the impact of thermal processing on its physical characteristics is an important consideration for dairy processors (Lewis and Deeth, 2009). Factors such as processing conditions, protein level and profile, pH, ionic strength and mineral composition can all significantly affect the heat stability and physical characteristics of the product (Singh, 2004; Jeswan Singh *et al.*, 2015). Heat treatment of dairy products at temperatures greater than 70 °C results in protein denaturation and aggregation of heat-labile whey proteins (Anema and Li, 2003; Chevallier *et al.*, 2016; Joyce *et al.*, 2017). This denaturation involves the unfolding of heat-labile whey proteins to expose reactive functional groups, such as the free thiol groups in β-lactoglobulin (β-lg). Subsequently, these functional groups can readily react with other denatured whey proteins, caseins, or κ-casein (κ-CN) at the casein micelle surface to form whey protein aggregates, whey protein-casein aggregates or whey protein/κ-CN complexes, respectively (Anema and Li, 2003; Donato and Guyomarc'h, 2009).

The type of aggregation in the system is informed by the type of proteins available. The casein to whey protein (CN:WP) ratio can have a significant impact on the type of heat-induced changes, such as particle size, viscosity and heat stability observed in dairy products (Beaulieu *et al.*, 1999; Singh, 2004; Donato and Guyomarc'h, 2009; Brodkorb *et al.*, 2016). For infant milk formula (IMF) with a total protein content of 1.5% (w/w), heat stability was found to be significantly affected by the CN:WP ratio (McSweeney *et al.*, 2004). Manipulation of the CN:WP ratio can alter the reaction rates of protein denaturation reactions (Anema *et al.*, 2006). Where a higher ratio of

whey protein resulted in increased quantities of whey protein aggregates (Beaulieu *et al.*, 1999; Yüksel and Erdem, 2005; Singh *et al.*, 2015), the addition of casein can improve heat stability, due to casein-whey protein aggregates exhibiting higher heat stability than whey protein aggregates (Patocka *et al.*, 1993).

The presence of casein can impact these heat-induced changes through a protective chaperone-like effect, resulting in the prevention of irreversible whey protein denaturation and aggregation during thermal processing and improvement of heat stability (O'Kennedy and Mounsey, 2006, Liyanaarachchi and Vasiljevic, 2018). α_s-, β-, and κ-Caseins have been shown to act like molecular chaperones; these protein molecules prevent unfolding, aggregating and precipitation of heat-labile proteins under unfavourable conditions by blocking the hydrophobic surfaces exposed from the denaturing proteins (Morgan *et al.*, 2005; Zhang *et al.*, 2005; Mounsey and O'Kennedy, 2010). It has been proposed that this chaperone-like mechanism is related to the higher charge density of casein-whey protein aggregates compared to native whey proteins, limiting interactions with other proteins (Gaspard *et al.*, 2017).

Preheat treatment can be applied to induce the aggregation of whey proteins and increase heat stability (Joyce *et al.*, 2016; Chevalier *et al.*, 2016; Gaspard *et al.*, 2017). The use of heat-induced whey protein aggregates has been used to improve the thermal stability of whey protein beverages (Ryan *et al.*, 2012; Ryan and Foegeding, 2015). The application of different preheat temperature-time combinations have been shown to alter the type of aggregation for heat-labile proteins due to partial unfolding at low temperatures, while the overall extent of whey protein denaturation remains the same and can result in this enhanced thermal stability (Williams *et al.*, 2008; Laiho et al., 2015). Joyce *et al.* (2016) varied preheat temperatures and calcium concentrations to

produce nanoparticulated whey protein aggregates with different surface reactivity, reducing the level of aggregation in a final infant milk formulation.

The physical characteristics of heat-treated dairy products can be influenced by preheat temperature selection and alteration of the CN:WP ratio. The aim of this study was to investigate if changes in these factors could produce dairy protein solutions at 4% (w/w) protein which have been minimally affected by heat treatment to significantly improve final product quality.

3.3. Materials and methods

3.3.1. *Materials and formulation*

Liquid skim milk was obtained from Moorepark Technology Ltd. (Fermoy, Cork, Ireland), with a composition of 3.31% protein, 0.05% fat, 4.78% lactose and 8.86% total solids. Whey protein isolate (WPI) was supplied by Davisco Foods International (Le Sueur, MN, USA), which had a composition of 91.8% protein, 0.21% fat, 2.03% ash, and <0.2% lactose. Milk permeate powder was used from Kerry Ingredients (Listowel, Co. Kerry, Ireland). A whey protein solution was made to match the total protein and solids content of the liquid skim milk. WPI and milk permeate were added to reverse osmosis water at 60 °C with a Silverson AX3 high shear mixer for 20 min (Silverson Machines Ltd, Bucks, England). The solution was stored overnight at 4 °C to facilitate complete rehydration and mineral equilibration. The liquid skim milk and whey protein solution were combined to produce four milk protein solutions with different CN:WP ratios of 80:20, 50:50, 20:80 and 0:100. The total protein and total solids content of each of the milk protein solutions was kept constant to equal that of the liquid skim milk. The skim milk proportions were 100, 62.57, 25 and 0% for 80:20, 50:50, 20:80 and 0:100 protein solutions, respectively, with the remainder being

composed of whey protein solution. The pH of each milk protein solution was checked to ensure proximity to 6.80 and no adjustment was required.

3.3.2. *Heat treatment*

Heat treatment of the milk protein solutions was carried out using a laboratory-scale thermal processing plant. Indirect heating was applied using an Armfield HTST/UHT Heat Exchanger Processing Unit FT74XTS (Armfield Limited, Hampshire, UK), consisting of two tubular heat exchangers for preheating and final heating operations and one tubular heat exchanger for cooling. A flowrate of 10.2 ± 0.14 L/h was applied and two preheat treatment conditions were used, HT I at 63 and HT II at 77 °C for 30 s, with final heating at 120 °C for 30 s followed by cooling to 16 °C. Residence time for the tubular heat exchangers was 5s. Samples of unheated and heated protein solutions were freeze-dried using a Labconco 12 L FreeZone 12 plus freeze drier (Labconco, Kansas City, MO, USA) at -40 °C to enable further viscosity analysis when reconstituted to a higher total solids content (O'Loughlin et al., 2015).

3.3.3. *Particle size analysis*

Particle size distribution of the milk protein solutions was analysed by with dynamic light scattering (DLS) using a Malvern Zetasizer Nano ZS (Malvern Instruments Ltd., Worcestershire, England). Samples were analysed at 25°C, with a refractive index of 1.45 and 1.33 for milk protein and dispersant, respectively. Samples were diluted in ultra-pure water (1 in 1000) and added to disposable polystyrene cuvettes for analysis.

3.3.4. *Colour*

Colour was measured using a Minolta Chroma Meter CR-400 colorimeter (Minolta Ltd., Milton Keynes, UK) and was expressed on the L*, a*, b* scale, as described by Kelleher *et al.* (2018a). Colour difference from unheated formulations, ΔE, was

calculated using the CIE76 Euclidean distance formula as described by (Morales and Jiménez-Pérez, 2001):

$$\Delta E = \sqrt{(L_2^* - L_1^*)^2 + (a_2^* - a_1^*)^2 + (b_2^* - b_1^*)^2} \quad 3.1$$

For ΔE, a value greater than 2.3 relates to a "just noticeable difference" in colour (Mokrzycki, and Tatol, 2011).

3.3.5. *Whey protein denaturation*

Soluble whey protein was determined using reverse phase-high performance liquid chromatography (RP-HPLC), as described by Kehoe *et al.* (2011) using a PolymerX 5 µm RP-1, 150 x 4.6 mm column (Phenomenex, Cheshire, UK). The mobile phases used for the separation were 0.1% TFA in water and 90% Acetonitrile 0.1% TFA.

3.3.6. *Viscosity*

Apparent viscosity was measured using an AR2000ex controlled-stress rheometer (TA Instruments, Crawley, UK), equipped with a 60mm parallel plate geometry at 25 °C. The procedure involved the samples being pre-sheared at 100 s^{-1} over 30 s, followed by equilibration at 0 s^{-1} for 1 min. The shear rate was then increased from 0.1 to 300 s^{-1} over 5 min, held at 300 s^{-1} for 1 min after which it was decreased from 300 to 0.1 s^{-1} over 5 min.

Freeze dried samples were rehydrated at higher total solids content than the original 8.86%, w/w, and the apparent viscosity was measured as above. The Power Law was applied to the shear rate versus shear stress measurements, in order to determine the rheological behaviour of the concentrated solutions at 20, 25 and 30% total solids:

$$\log \sigma = \log K + n \log \gamma \quad 3.2$$

The value of the flow behaviour index, n, a dimensionless number, signifies how Newtonian a fluid is, given that Newtonian fluids have a value of 1. Samples with values for *n* greater than 1 are considered dilatant or shear-thickening, while samples with n values less than 1 are considered pseudoplastic or shear-thinning (Kelleher *et al.*, 2018b).

3.3.7. *Accelerated storage stability*

The physical stability of the milk protein solutions was analysed under accelerated conditions using an analytical centrifuge LUMiSizer 6112 (L.U.M. GmbH, Berlin, Germany) with SepView 4.1 software to determine the impact of creaming or sedimentation. Samples (0.4 mL) were filled into PC100-131XX polycarbonate cells using a wide-bore needle (20 gauge) and centrifuged at 2300g for 3 h at 25 °C (Chen and O'Mahony, 2016). The system measures the intensity of transmitted near infrared (NIR) light as a function of time and position across the sample in the cell. The software uses integration to measure the change in transmission over time with integration limits of 108.7 to 130 mm, which is then used to calculate the instability index for each milk protein solution.

3.3.8. *Statistical analysis*

Heat treatment was carried out in duplicate. Minitab® 17 (Minitab Ltd., Coventry, UK) statistical analysis package was used to carry out one-way ANOVA with Tukey post hoc analysis. A non-parametric test, the Wilcoxon signed rank test, was performed on viscosity data for concentrated protein solutions, using Sigmastat software (SPSS Inc., San Jose, CA, USA).

3.4. Results

3.4.1. *Particle size distribution*

The effect of casein to whey protein ratio on particle size distribution (PSD) of the protein solutions is shown in Fig. 3.1. The PSD of the milk protein solutions was influenced by the protein profile of the solutions. The whey protein solution (0:100) had a bimodal distribution, which changed to a broadly mono-modal distribution for 20:80 and became distinctly mono-modal at higher levels of casein ($p < 0.001$). This bi-modal distribution for the 0:100 unheated control solution is likely related to the formation of soluble aggregates during prior thermal processing in the production of the powder. Heat treatment significantly increased the average particle size of the protein solutions from that of the unheated control for each respective protein solution, with the exception of the 80:20 sample (Table 3.1). The impact of heat treatment on particle size (increase in particle size) decreased with increasing casein content.

Preheat temperatures, HT I and II, resulted in significantly different average particle sizes for non-casein 0:100 solutions, with the higher preheat temperature, HT II, giving a smaller average particle size than the lower preheat temperature, HT I. This is likely due to the impact of preheat temperature-time combinations, in which the type of protein aggregation is altered and smaller aggregates are produced. In contrast, the particle size of heat-treated casein-containing solutions (20:80, 50:50 and 80:20) was unaffected by preheat temperature. The large increase in particle size with heat treatment, seen for a CN:WP ratio of 20:80 for both HT I and II, may be attributed to the formation of large whey-casein aggregates with the introduction of casein into the system (Fig. 3.1).

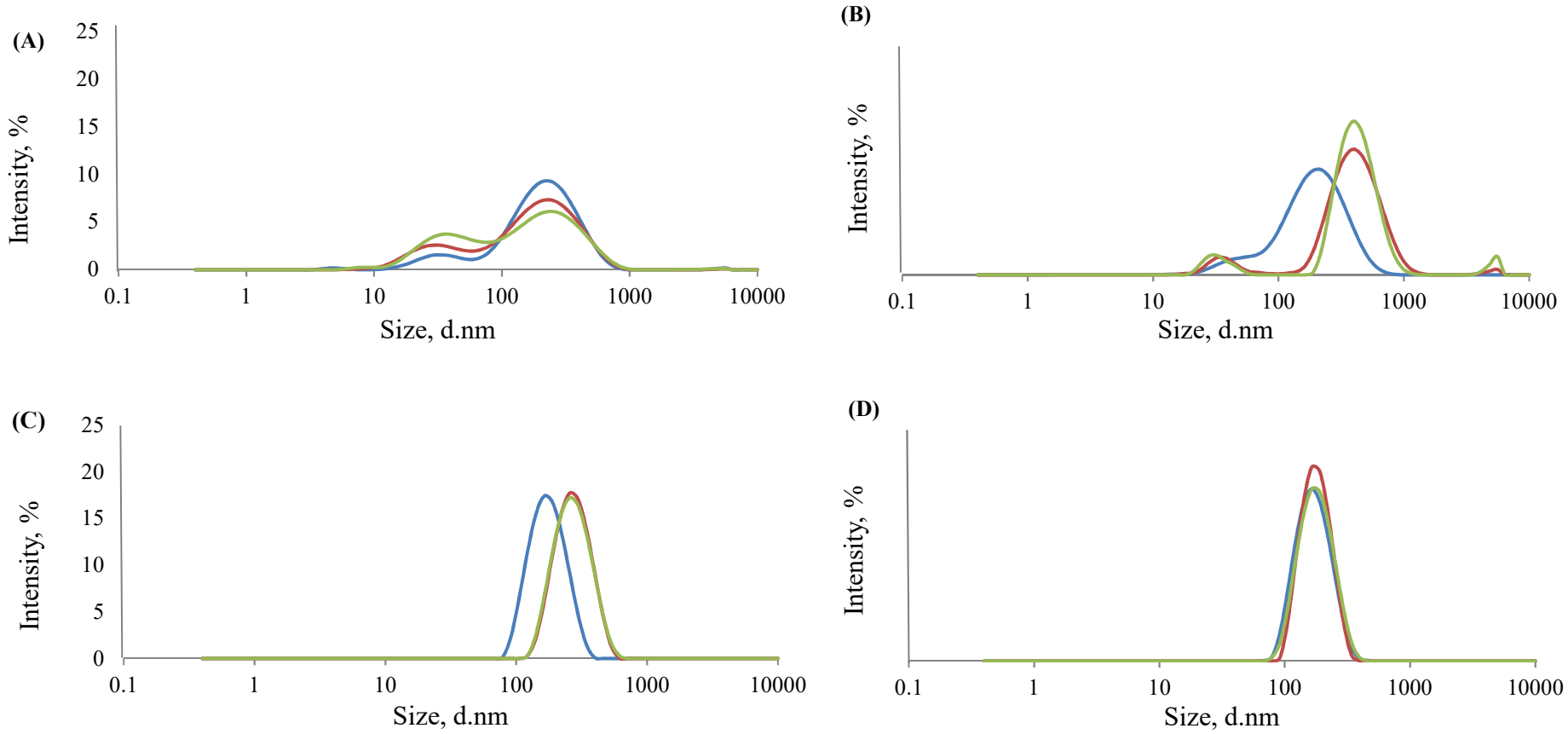

Fig. 3.1. Particle size distributions of milk protein solutions with casein to whey protein ratios of (A) 0:100, (B) 20:80, (C) 50:50 and (D) 80:20 for unheated control (—), heat treatment I at 63°C (—), and heat treatment II at 77°C (—).

3.4.2. *Colour*

The colour of each protein solution, expressed as L*, a* and b* values, was significantly affected by the CN:WP ratio ($p < 0.001$). The lightness value, L*, of solutions with a 50% or greater casein proportion was significantly higher than for those with a high proportion of whey protein. For each of the solutions, the lightness generally increased upon heat treatment; however, this increase was not significant for all protein solutions (Table 3.1; $p > 0.05$). Preheat treatment had no significant effect on L* value ($p > 0.05$). Likewise, the applied heat treatment did not significantly affect the a* value, redness-greenness, of the protein solutions ($p > 0.05$). However, the b* value, yellowness-blueness, was significantly affected by heat treatment, reducing upon heating for the whey protein solution, 0:100, while b* increased upon heat treatment of the casein-containing 20:80, 50:50 and 80:20 solutions.

Euclidean colour difference, ΔE, was significantly affected by the CN:WP ratio ($p < 0.001$) but not by the selected heat treatment ($p > 0.05$). The largest ΔE was found for the heat-treated 50:50 solutions (average ΔE of 31.2), which was significantly greater than for all other solutions, mainly reflecting the significant effect of change b* value for the 50:50 solution. The colour difference for heat-treated 80:20 solutions was in the 'just noticeable difference' region, while 0:100 and 20:80 solutions more evident colour changes with average ΔE values of 8.79 and 8.69, respectively, the latter result showing a greater sensitivity of ΔE versus the separate parameters L*, a* and b*.

3.4.3. *Whey protein denaturation*

Denaturation of α-lactalbumin (α-la) was significantly affected by level of casein present in the protein solution (Table 3.2; $p < 0.001$), with increasing casein content resulting in a significantly greater retention of native α-la for solutions

Table 3.1. Physical characteristics of milk protein solutions with different casein to whey protein (CN:WP) ratios of 0:100, 20:80, 50:50 and 80:20[1].

CN:WP Ratio	Heat Treatment[2]	pH	Apparent viscosity (300 1/s)	Average Particle Size	Colour				Instability Index
		-	mPa.s	d.nm	L*	a*	b*	ΔE	(%/h)
0:100	Control	6.81 ± 0.02^{a}	1.54 ± 0.05^{a}	116 ± 2.99^{a}	32.4 ± 1.30^{a}	-1.11 ± 0.20^{a}	3.39 ± 0.06^{a}	-	4.30 ± 0.27^{a}
0:100	HT I	6.65 ± 0.04^{b}	1.67 ± 0.13^{a}	96 ± 2.27^{b}	33.5 ± 0.66^{a}	-1.38 ± 0.70^{a}	0.61 ± 0.62^{b}	9.06 ± 3.82^{a}	7.14 ± 0.07^{b}
0:100	HT II	6.66 ± 0.03^{b}	1.69 ± 0.17^{a}	79 ± 2.96^{c}	33.2 ± 0.73^{a}	-0.69 ± 0.03^{a}	0.68 ± 0.74^{b}	8.51 ± 4.26^{a}	6.47 ± 0.18^{b}
20:80	Control	6.87 ± 0.01^{a}	1.66 ± 0.11^{a}	136 ± 30.2^{b}	42.3 ± 6.50^{a}	-2.13 ± 1.17^{a}	-1.34 ± 2.35^{a}	-	11.6 ± 0.65^{a}
20:80	HT I	6.76 ± 0.10^{a}	1.79 ± 0.12^{a}	283 ± 28.8^{a}	46.3 ± 2.66^{a}	-1.68 ± 0.72^{a}	-0.33 ± 0.30^{a}	8.66 ± 1.68^{a}	45.1 ± 0.91^{b}
20:80	HT II	6.77 ± 0.08^{a}	1.78 ± 0.09^{a}	318 ± 22.2^{a}	46.2 ± 2.60^{a}	-1.67 ± 0.66^{a}	-0.38 ± 0.37^{a}	8.71 ± 1.47^{a}	49.0 ± 3.90^{b}
50:50	Control	6.78 ± 0.02^{a}	1.70 ± 0.06^{a}	164 ± 5.84^{b}	66.2 ± 3.36^{a}	-4.13 ± 0.95^{a}	-4.33 ± 0.54^{a}	-	34.4 ± 0.94^{a}
50:50	HT I	6.69 ± 0.06^{a}	1.55 ± 0.20^{a}	249 ± 5.84^{a}	71.6 ± 6.41^{a}	-3.20 ± 0.91^{a}	0.76 ± 1.36^{b}	31.8 ± 11.4^{a}	67.2 ± 3.59^{b}
50:50	HT II	6.67 ± 0.00^{a}	1.69 ± 0.01^{a}	245 ± 7.29^{a}	70.8 ± 5.59^{a}	-3.06 ± 0.88^{a}	0.72 ± 1.19^{b}	30.5 ± 8.76^{a}	72.6 ± 4.33^{b}
80:20	Control	6.71 ± 0.01^{a}	1.89 ± 0.10^{a}	160 ± 2.51^{a}	75.2 ± 3.65^{a}	-4.76 ± 1.09^{a}	-2.54 ± 0.64^{a}	-	41.4 ± 1.46^{a}
80:20	HT I	6.57 ± 0.04^{b}	1.76 ± 0.08^{a}	170 ± 3.75^{a}	77.1 ± 5.81^{a}	-4.75 ± 1.03^{a}	-2.35 ± 1.22^{a}	2.04 ± 2.38^{a}	40.9 ± 1.36^{a}
80:20	HT II	6.54 ± 0.01^{b}	1.87 ± 0.04^{a}	168 ± 4.31^{a}	77.3 ± 5.90^{a}	-4.74 ± 1.00^{a}	-2.34 ± 1.21^{a}	2.32 ± 2.47^{a}	40.7 ± 1.07^{a}

[1] Values presented in the table are the mean ± standard deviation of duplicate measurements; for each CN:WP ratio solution, values within a column not sharing a common superscript differ significantly ($p < 0.05$) as determined by one-way ANOVA.

[2] The different heat treatments applied are control (unheated), HT I (63 °C preheat and 120 °C final heat) and HT II (77 °C preheat and 120 °C final heat).

with 50% or more casein as a proportion of total protein. At a casein to whey protein ratio of 80:20, 45.22% of native α-la in the unheated solution was retained, while the whey protein 0:100 solution had only 3.17% of native α-la, on average, after heat treatment. The application of different preheat temperatures did not significantly affect the level of α-la denaturation in the protein solutions ($p > 0.05$).

Table 3.2. Native whey proteins α-lactalbumin, β-lactoglobulin B and β-lactoglobulin A as determined by reverse phase HPLC, presented as a percentage of the native protein concentration in the respective unheated control samples[1].

CN:WP Ratio	Heat Treatment[2]	α-lactalbumin	β-lactoglobulin B	β-lactoglobulin A
		%	%	%
0:100	HT I	3.11 ± 0.04^{c}	5.44 ± 1.01^{a}	5.27 ± 0.26^{a}
	HT II	3.22 ± 0.19^{c}	4.72 ± 1.31^{a}	4.70 ± 0.86^{a}
20:80	HT I	5.59 ± 0.98^{bc}	6.54 ± 1.79^{a}	6.44 ± 0.17^{a}
	HT II	4.24 ± 0.48^{c}	4.48 ± 1.28^{a}	4.02 ± 0.30^{a}
50:50	HT I	14.7 ± 2.50^{bc}	6.30 ± 0.48^{a}	5.90 ± 0.15^{a}
	HT II	11.2 ± 0.79^{b}	3.59 ± 0.03^{a}	3.78 ± 0.03^{a}
80:20	HT I	45.0 ± 3.09^{a}	4.27 ± 0.90^{a}	4.69 ± 1.36^{a}
	HT II	45.5 ± 6.15^{a}	4.23 ± 0.88^{a}	4.67 ± 1.14^{a}

[1]Values presented in the table are the mean ± standard deviation of duplicate measurements; values within a column not sharing a common superscript differ significantly ($p < 0.05$).
[2] The different heat treatments applied are control (unheated), HT I (63 °C preheat and 120 °C final heat) and HT II (77 °C preheat and 120 °C final heat).

Heat-labile β-lactoglobulin (β-lg) A and B whey protein fractions were heavily denatured by the extensive heat treatment processes for each of the protein solutions, with average native levels reduced to 4.93 % and 4.95 % for β-lg A and B, respectively (Table 3.2). No significant differences in β-lg A or B denaturation were found, either with respect to preheat temperature or casein to whey protein ratio ($p > 0.05$).

3.4.4. *Viscosity*

Overall, the apparent viscosity of the protein solutions was not significantly affected by heat treatment (Table 3.1; $p > 0.05$). In addition, the apparent viscosity for each

CN:WP ratio was not significantly different, indicating that the CN:WP ratio of the solutions had no impact on viscosity. However, viscosity differences for dilute systems can be difficult to determine to a statistically significant degree (Sutariya *et al.*, 2017). To improve the sensitivity in evaluating viscosity changes, theheat-treated protein solutions were freeze-dried and reconstituted at higher total solids levels for further viscosity analysis (Table 3.3). A non-parametric test revealed a statistically significant trend regarding the effect of preheat temperature for all concentrated protein solutions, where HT II consistently resulted in a higher apparent viscosity than HT I ($p < 0.01$). The difference between HT I and II was greater for protein solutions containing a higher proportion of whey protein, 0:100 and 20:80. As with denaturation of α-la, the CN:WP ratio had a significant impact on the resistance of the solution to changes in physical properties due to heat treatment at 20% total solids. The 0:100 and 20:80 solutions solution had increased levels of protein denaturation and broader particle size distributions, indicating differences in the aggregates formed, compared to the 50:50 and 80:20 solutions. It would be reasonable that these factors would influence viscosity.

The flow behaviour of the concentrated solutions was also investigated through the application of the Power Law. The flow behaviour index, *n*, indicates the closeness of the fluid to Newtonian behaviour (n=1). At 20% total solids, the protein solutions can be considered relatively Newtonian in behaviour with an average *n* value of 0.91 (Table 3.3). While no significant difference was determined ($p > 0.05$), the *n* value decreased as the total solids concentration increased. This indicates that some solutions, particularly for the 20:80 solution at 25% total solids, develop a shear-thinning

Table 3.3. Rheological behaviour of the freeze-dried protein solutions after reconstitution to 20, 25 and 30% total solids at 300 1/s and flow behaviour index determined using the Power Law.[1]

Dairy protein solutions			Apparent viscosity, 300 s^{-1}	Flow behaviour index, n
Total Solids %	CN:WP Ratio	Heat Treatment	mPa.s	-
20%	0:100	HT I	$12.0 \pm 0.95^{d.A}$	$0.96 \pm 0.02^{a.A}$
		HT II	$16.7 \pm 7.24^{d.A}$	$0.94 \pm 0.04^{a.A}$
	20:80	HT I	$12.5 \pm 3.65^{d.A}$	$0.94 \pm 0.06^{a.A}$
		HT II	$20.3 \pm 14.0^{d.A}$	$0.92 \pm 0.05^{a.A}$
	50:50	HT I	$7.30 \pm 1.10^{d.A}$	$0.94 \pm 0.02^{a.A}$
		HT II	$7.58 \pm 0.52^{d.A}$	$0.95 \pm 0.00^{a.A}$
	80:20	HT I	$6.11 \pm 0.55^{d.A}$	$0.92 \pm 0.03^{a.A}$
		HT II	$6.64 \pm 1.25^{d.A}$	$0.83 \pm 0.03^{a.A}$
25%	0:100	HT I	-[2]	-
		HT II	-	-
	20:80	HT I	$121.8 \pm 0.58^{ab.A}$	$0.67 \pm 0.15^{a.A}$
		HT II	$172.3 \pm 31.8^{a.A}$	$0.51 \pm 0.22^{a.A}$
	50:50	HT I	$16.85 \pm 3.63^{d.B}$	$0.89 \pm 0.05^{a.A}$
		HT II	$17.02 \pm 0.95^{d.B}$	$0.95 \pm 0.00^{a.A}$
	80:20	HT I	$11.35 \pm 0.82^{d.B}$	$0.88 \pm 0.02^{a.A}$
		HT II	$12.52 \pm 2.09^{d.B}$	$0.66 \pm 0.26^{a.A}$
30%	0:100	HT I	-	-
		HT II	-	-
	20:80	HT I	-	-
		HT II	-	-
	50:50	HT I	$35.29 \pm 3.90^{cd.A}$	$0.91 \pm 0.02^{a.A}$
		HT II	$42.37 \pm 1.73^{cd.A}$	$0.86 \pm 0.03^{a.A}$
	80:20	HT I	$23.83 \pm 2.38^{d.B}$	$0.85 \pm 0.03^{a.A}$
		HT II	$24.72 \pm 5.63^{d.B}$	$0.61 \pm 0.32^{a.A}$
35%	0:100	HT I	-	-
		HT II	-	-
	20:80	HT I	-	-
		HT II	-	-
	50:50	HT I	-	-
		HT II	-	-
	80:20	HT I	$58.17 \pm 14.6^{cd.A}$	$0.79 \pm 0.04^{a.A}$
		HT II	$84.83 \pm 42.8^{bc.A}$	$0.78 \pm 0.01^{a.A}$

[1]Values presented in the table are the mean ± standard deviation of duplicate measurements; values within a column not sharing a common superscript differ significantly ($p < 0.05$), where upper- and lower-case letters indicate that one-way ANOVA was performed on the individual total solids groupings, and entire set, respectively.

[2] Dashes (-) in the table represent concentrations not tested as the reconstituted concentrated protein solution was very viscous.

rheological behaviour with concentration and is consistent with previous reports for dairy systems (Morison *et al.*, 2013; Anema *et al.*, 2014). This shear-thinning is the result of the increased shear rate deforming or rearranging the particles, resulting in lower flow resistance (Bylund, 2003).

3.4.5. *Accelerated storage stability*

The stability of the protein solutions under accelerated conditions solution varied significantly with the CN:WP ratio, with stability increasing with decreasing proportion of casein (Table 3.1; $p < 0.001$). For unheated solutions, those with a higher proportion of whey protein, 0:100 and 20:80, resulted in higher levels of storage stability compared to those with an equal or greater proportion of casein, 50:50 and 80:20. For 0:100, 20:80 and 50:50 protein solutions, i.e. >50% of protein in the form of whey protein, heat treatment resulted in a significant decrease in accelerated storage stability ($p < 0.05$), while the 80:20 solution (having the lowest stability among the unheated formulations) had similar levels of stability for the unheated, HT I and HT II treatments. The choice of preheat treatment had no effect on level of stability ($p > 0.05$).

3.5. Discussion

Different preheat temperatures have been shown to alter the degree of whey protein denaturation and improve heat stability (Ryan and Foegeding, 2015; Chevalier et al., 2016; Gaspard et al., 2017). In this study, the application of two different preheat temperatures, 63 and 77 °C, impacted the average particle size of whey protein solutions, 0:100, with HT II resulting in a significantly smaller particle size than the lower level treatment, HT I. Apparent viscosity was shown to be impacted by preheat temperature when the protein solutions were concentrated, with higher heat treatment

temperatures resulting in a higher apparent viscosity. These results are in agreement with the findings for concentrated skim milk reported by Sutariya *et al.* (2017). Milks which go forward to evaporation operations can pose technical difficulties due to increased viscosity. Altering the preheat temperature to improve viscosity of concentrated milk solutions could prove to be a useful way of reducing the impact of such issues.

The protein profile, CN:WP ratio, had a substantial impact on the physical characteristics of heat-treated protein solutions. One of the most significantly impacted characteristics was whey protein denaturation level after heat-treatment. Denaturation of heat-labile whey proteins has been shown to commence above ~62°C and ~75 °C for α-la and β-lg, respectively (Fitzsimons *et al.*, 2007). While β-lg was extensively denatured for all solutions, the proportion of casein present had a significant impact on the level of native α-la. Indeed, the addition of casein provided a statistically significant protective effect against α-la denaturation for the 80:20, 50:50 and the HT I-treated 20:80 protein solutions Akkerman (2014) reported similarly low levels of α-la denaturation for skim milk heated using a plate heat exchanger at 115 and 130 °C. Our findings are also in agreement with the work of Beaulieu *et al.* (1999), who reported increased levels of α-la denaturation was observed with increased concentrations of whey protein. Therefore, the presence of casein seemed to have a beneficial protective effect on α-la towards heat-induced changes and was greatly dependent on the level of casein in the system. However, this protective effect of casein was not applicable to β-lg, which had similar denaturation levels with or without the presence casein.

Changes in the b* value have been used to monitor protein aggregation and Maillard browning in dairy systems, with the latter reaction in dairy systems involving

interaction of the reducing sugar, lactose, with free amino groups (Morales and van Boekel, 1998; Arena *et al.*, 2017). Conjugation of whey proteins by Maillard reactions can be limited due to irreversible heat-induced aggregation, which reduces the accessibility of carbonyl groups to amino groups required for Maillard reactions compared to caseins (O'Mahony at al., 2017). The increase in b* values for casein-containing solutions may be due to an increased availability of free amino acids, with high levels of protein denaturation exposing and activating amino acid residues for participation in Maillard reactions (Fox *et al.*, 2015). The colour change and impact on flavour associated with Maillard browning of heat-treated milks can have a negative impact on consumer perception and product acceptability.

The largest average particle size was seen for heat-treated 20:80 protein solutions, due to the formation of large aggregates for both HT I and II. The presence of a low proportion of casein, and substantial whey protein denaturation, likely resulted in the formation of large whey-casein aggregates, as seen through increases in the average particle size for the heat-treated 20:80 solution (Gaspard *et al.*, 2017; Liyanaarachchi and Vasiljevic, 2018). This suggests that having 20% of the total protein as casein may not be sufficient to impart the protective chaperone effect, producing larger protein aggregates compared to the other protein solutions. Previous studies (Anema *et al.*, 2003; Donato and Guyomarc'h, 2009; Joyce *et al.*, 2017) have shown whey proteins to associate with casein micelles *via* β-lactoglobulin/κ-casein complexes during heat treatment. Therefore, in 20:80 WP:CN solutions there was a large amount of whey protein denaturation but also complexation with casein micelles and thus resulted in a larger average particle size (Fig. 3.1B). Interestingly, solutions with a higher proportion of casein, e.g. 80:20, showed no significant change in particle size due to

heating, indicating improved thermal stability and the formation of few protein aggregates compared to the other protein solutions.

3.6. Conclusion

The preheat temperatures investigated in the study had little impact on the physical characteristics of the heat-treated protein solutions, only affecting the apparent viscosity of protein solutions at higher solids levels. Whereas alterations to the CN:WP ratio had a significant impact on the final product quality of the heat-treated solutions. The extent of α-la denaturation was significantly reduced with an increasing proportion of casein, even for solutions with a relatively low level (i.e., 20% of total protein). An increasing proportion of casein also reduced the change in average particle size due to heat-treatment. Observable changes in colour due to heat-treatment were found for the casein-containing 50:50 solution, which may negatively impact consumer perception and may need to be considered in product development. Alteration of the CN:WP ratio can be utilised to significantly influence the product quality, with the addition of casein reducing physical changes due to heat-treatment for dairy protein solutions.

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Chapter 4.

The effect of direct and indirect heat treatment on the attributes of whey protein beverages

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4.1. Abstract

Thermal processing of ready-to-drink high protein beverages can have a substantial impact on the physical and sensory properties of the final product for long-life milks like extended shelf life (ESL) and ultra-high temperature (UHT) processed products. Direct and indirect heat treatment technologies were applied to whey protein isolate (WPI) - based beverages containing 4, 6 or 8 % (w/w) protein. Lower levels of protein denaturation (66-94 %) were observed using direct heating compared to indirect heating (95-99 %) across protein levels and heating temperatures (121 and 135 °C final heat). Direct heat treatment resulted in significantly lower viscosity and less extensive changes to the volatile profile, compared to indirect heat treatment. Overall, the application of direct and indirect heat treatment to WPI solutions resulted in significantly different final products in terms of appearance, physical characteristics and volatile profile, with direct heating resulting in many enhanced properties compared to conventional indirect heat treatment.

4.2. Introduction

Nutritional beverages are a rapidly growing market segment, with sales increasing by an average of approximately 5 % annually (Cochrane *et al.*, 2012; Chen and O'Mahony, 2016). These products can be formulated to cater for a variety of consumer needs such as functional sports foods for high performance athletes and body-builders, meal replacement drinks for dietetic nutrition, and low-sugar drinks for diabetic patients (Beecher *et al.*, 2008; Jelen, 2009; Shiby, 2013). When developing protein beverages, whey proteins are commonly used as a protein source due to their excellent nutritional qualities, bland flavour, ease of digestibility and functionality in beverage systems (Rittmanic, 2006). Formerly considered a waste by-product of cheese and casein production, whey protein has become highly valued for its nutritional and functional properties (Evans, 1980; Mulvihill and Ennis, 2003; Fitzsimons *et al.*, 2007; Smithers, 2008; Boland, 2011). However, technological processes used in dairy-based beverage manufacture may impair the high nutritional value of whey proteins, whereby protein denaturation and aggregation and loss of solubility decrease protein digestibility and the bioavailability for enzymatic digestion (Pellegrino, 2013). As a result, selection of thermal processing technology is an important factor affecting the level of protein denaturation and nutritional value of products, in addition to reducing aggregate-related storage stability issues in long-life products, such as increases in viscosity, turbidity and sedimentation (Villumsen *et al.*, 2015a and b, Le *et al.*, 2016).

Typical heat treatment processes used during manufacture of whey protein beverages are in the extended shelf life (ESL) heat treatment range (120 - 135°C for 2-4 s) or ultra-high temperature (UHT) range (135 – 145°C for 2-4 s) (Rysstad and Kolstad, 2006; Britz and Robinson, 2008; Deeth and Lewis, 2016). There are two classical

modes of high temperature short time (HTST) heating, i.e., indirect and direct heating, used for the commercial sterilisation of milk and milk products (Deeth and Lewis, 2016; Roux *et al.*, 2016). Indirect systems, using systems like tubular and plate heat exchangers, promote heat transfer across an interface while, for direct systems, like injection and infusion, the heating medium, steam, is in direct contact with the product and subsequently removed through flash cooling (Hsu, 1970; Burton, 1994; Schroyer, 1997; Lewis and Heppell, 2000). The heat transfer interface of indirect heating systems reduces the heat transfer rate and localised heating at the interface can result in higher levels of protein denaturation and fouling compared to direct systems (Murphy, 2011, Karayannakidis *et al.*, 2014, Akkerman *et al.*, 2016). In direct heating systems, almost instantaneous heating is achieved due to the mixing of the heating medium and product. This method involves a more thermodynamically efficient and rapid rate of heat transfer than indirect heating, as it makes use of the latent heat of evaporation as the steam condenses, resulting in reduced residence time and a lower thermal load imparted on the product, where the thermal load is a loose term referring to thermal history of a heating (or heating and cooling) process; it can refer to area under a temperature/time curve as shown in Fig 4.1c or as transformed by equation 4.1. (Datta *et al.*, 2002; Britz and Robinson, 2008; Karayannakidis *et al.*, 2014; Dickow *et al.*, 2012b Lee *et al.*, 2017).

In a number of studies direct heat treatment technology led to a reduced level of whey protein denaturation compared to indirect heating for skim milk (Lyster *et al.*, 1971; Akkerman *et al.*, 2016; Lee *et al.*, 2017) and whey protein concentrate (Dickow *et al.*, 2012a). However, direct treatments are also reported to result in a greater average particle size and sediment formation compared to indirect systems, due to the reduced area of thermal transfer surfaces in direct systems for deposition of aggregates (Burton,

1968; Datta *et al.*, 2002; Malmgren *et al.*, 2017). The studies imply that aggregates that would generally adhere to hot surfaces and be found in fouling material during traditional indirect processing are still present in the final product. The rapid cooling in direct heating can remove volatiles in milk such as dissolved oxygen, heat-induced sulphur volatiles and other volatiles, in addition to removing excess water, resulting in less heat-induced flavour changes (Deeth and Lewis, 2016; Lee *et al.*, 2017). Previous studies have identified direct heating processes as the best technological option to limit thermally-induced changes in milks (Van Asselt *et al.*, 2008; Roux *et al.*, 2016).

The heat treatment technology employed in dairy beverage production can have a significant impact on the taste, physical stability, and shelf life of the product. Little has been published in relation to the heat treatment of high protein whey solutions using direct heat treatment technology (Dickow *et al.*, 2012a) or the comparison of direct and indirect technologies. The aim of this study was to investigate the impact of direct and indirect heat treatment technology for two high temperature treatments (70/121 °C and 80/135 °C with preheat and final holding time of 30 s and 2 s, respectively), monitoring changes in selected physicochemical characteristics of high protein ready-to-drink whey protein beverages from the unheated controls and determining if either technology produced significantly enhanced product quality.

4.3. Materials and methods

4.3.1. *Materials and formulation*

Model whey-protein beverages were formulated at protein concentrations of 4, 6 and 8% w/w, reflective of current market product protein concentrations, using whey protein isolate (BiPro®), supplied by Davisco Foods International (Le Sueur, MN,

USA), which had a composition of 91.8% protein, 0.21% fat, 2.03% ash, and <0.2% lactose. The WPI powder were reconstituted in 150L batches using reverse-osmosis water heated to 45°C, to aid solubilisation of the ingredients. A YTRON ZC powder induction unit (YTRON Process Technology GmbH, Bad Endorf, Germany), consisting of a high-shear, rotor-stator mixer connected to a recirculation pump, was used for ingredient induction with a 20 min recirculation time. The dispersion was stored in a tank equipped with an impeller and stirred at a low speed overnight at 4°C. The pH was adjusted to pH 6.8 using 0.1 M HCl or KOH, as required, before and after overnight storage.

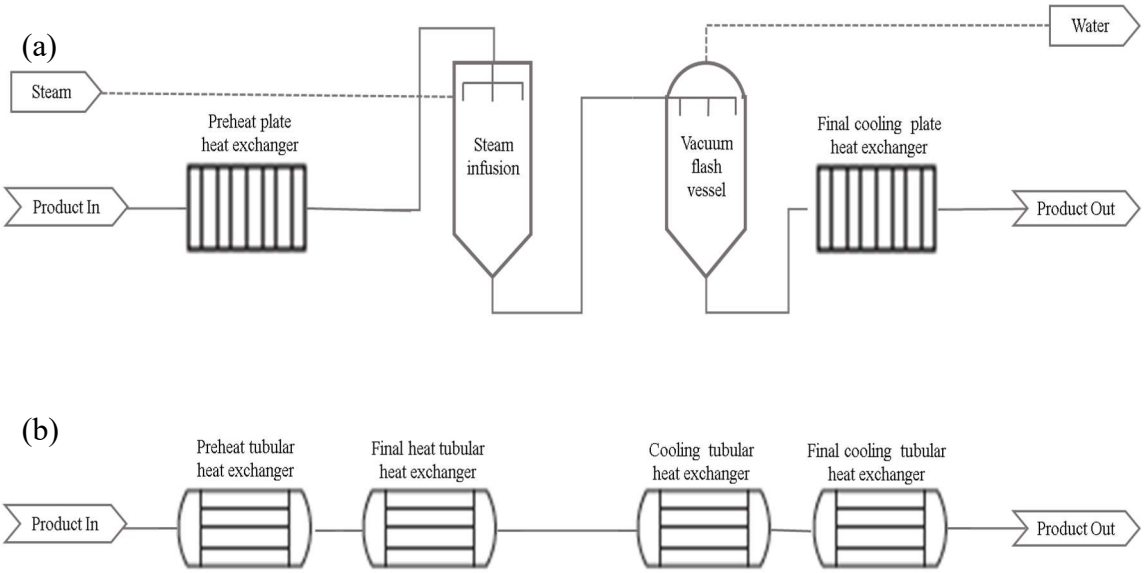
4.3.2. *Heat treatment*

Two pilot-scale thermal processing plants were used to carry out direct and indirect heat treatment of the WPI dispersions at 100 L/h. Direct heating was applied using a UHT steam infusion pilot plant 422463 (APV, Silkeborg, Denmark), which consists of a plate heat exchanger for preheating followed by steam infusion and flash cooling vessel, and a plate heat exchanger for final cooling (Fig. 4.1a). The residence time for the plate preheat, steam infusion and flash cooling operations were taken as 60, 3 and 1s, respectively. Indirect heating was applied using a MicroThermics tubular UHT pilot plant (MicroThermics, NC, USA), consisting of two tubular heat exchangers for preheating and final heating operations and two tubular heat exchangers for initial and final cooling operations (Fig. 4.1b). The residence time within a tubular heat exchanger for the MicroThermics system is 30s at 100 L/hr. Both the direct and indirect pilot plants were used with a preheat holding tube time of 30 s and a final heat holding time of 2 s (Fig. 4.1c). Two types of heating conditions were applied to the WPI dispersions using the direct and indirect pilot plants; 70°C preheat with 121°C final heat, and 80°C preheat with 135°C final heat. These temperature combinations

are commonly used for extended-shelf-life (ESL) and ultra-heat-treatment (UHT) processes, respectively (Burton, 1994; Bylund, 1995; Rysstad and Kolstad, 2006). The temperature combinations used will be referred to as ESL (70/121 °C) and UHT (80/135 °C) to ease description. A calculation of the F_0 value, a bacterial lethality index, of the two heat treatment technologies was completed to evaluate if the differences in thermal load, due to heating and cooling times, significantly impacted the predicted decimal reduction of bacterial organisms and comparability of the systems. The F_0 was determined using the following equation:

$$F = \int_0^\infty 10^{(\theta-\theta_{ref})/z} \,.\, \mathrm{dt} \qquad 4.1$$

Where the reference temperature, θ_{ref}, is 121.1 ºC and the z-value is 10 ºC as determined for *Clostridium botulinum* spores (Lewis and Heppell, 2000). Using this method of analysis, the F_0 values were determined to be reasonably comparable at 0.50 and 0.51 for ESL treatment, and 12.66 and 12.81 for UHT treatment for the direct and indirect heat treatments, respectively.



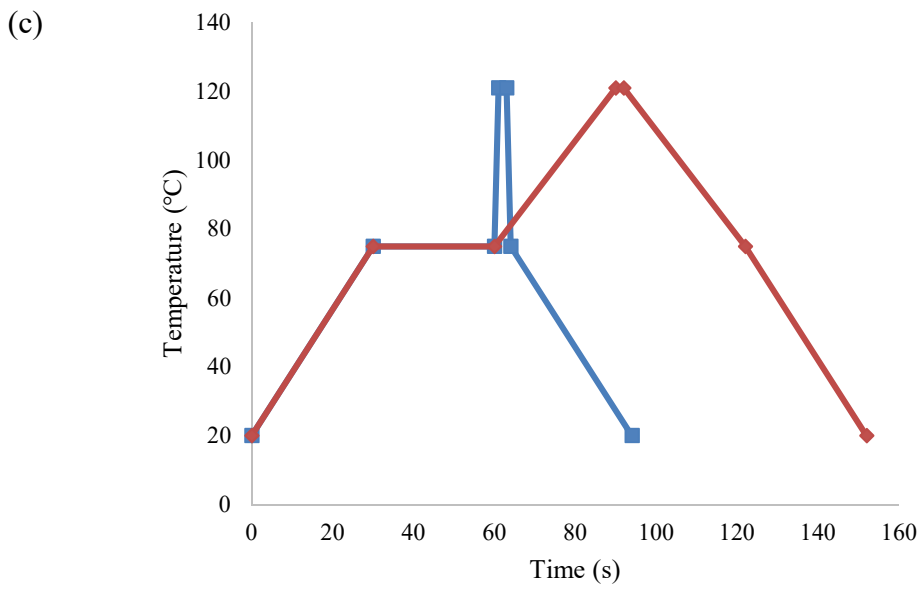


Fig. 4.1. Process flow diagram of (a) direct and (b) indirect heat treatment plants and (c) time-temperature heating and cooling profiles of indirect (tubular heat exchanger) (—) and direct (steam infusion or injection) (—) heat treatment technologies.

4.3.3. *Particle size analysis and molecular weight distribution*

Particle size distribution data of whey protein dispersions was determined using dynamic light scattering (DLS) with a Malvern Zetasizer Nano ZS instrument (Malvern Instruments Ltd., UK). Samples were dispersed in ultra-pure water for analysis in polystyrene disposable cuvettes. A refractive index of 1.45 was used for protein samples, while a refractive index of 1.330 was used for the dispersant. All samples were analysed at a temperature of 25°C.

Size-exclusion high-performance liquid chromatography (SE-HPLC) was used to monitor the formation of heat-induced aggregates by determining the molecular weight (Mw) profile of the samples as described by Buggy *et al.* (2016). The HPLC system used consisted of a Waters 2695 separation module with a Waters 2487 dual-wavelength detector at 280 nm, controlled using Waters Empower® software (Waters, Milford, Massachusetts, USA) using two columns in series (TSKgel

G2000SWXL and G3000SWXL, 7.8 mm ID, 30 cm length, 5 μm particle size, Tosoh Biosciences LLC, USA) with a guard column (TSKgel SWXL, 6 mm ID × 4 cm length, 7 μm particle size).

4.3.4. *Colour analysis*

In order to investigate potential heat-induced changes in colour due to aggregation of heat labile proteins colour measurements were carried out before and after heat treatment. The colour of each dispersion was measured and expressed as L*, a* and b* values using a Minolta Chroma Meter CR-400 colorimeter (Minolta Ltd., Milton Keynes, UK). The L* value indicates lightness, a* values indicate redness-greenness, b* values indicate yellowness-blueness. Samples were loaded into a disposable cuvette and placed in front of a white calibration plate (L*, a*, b*) before measurement in triplicate.

4.3.5. *Viscosity*

Viscosity can impact final product acceptability for consumers, and was measured using an ARG2 controlled-stress rheometer (TA Instruments, Crawley, UK) equipped with concentric cylinder geometry at 25 °C. The procedure involved the samples being pre-sheared at 500 s^{-1} for 1 min followed by equilibration at 0 s^{-1} for 1 min, to neutralise the short-term rheological history of the formulations. The shear rate was then increased from 5 to 500 s^{-1} over 2 min, held at 500 s^{-1} for 1 min then decreased from 500 to 5 s^{-1} over 2 min (Murphy *et al.*, 2013).

4.3.6. *Protein analysis and total solids measurement*

The total solids content of the dispersions was measured using a Smart System 5, Smart Trac (CEM Corporation, Matthews, NC, USA).

Determination of total protein content of samples was carried out using the Kjeldahl method of analysis (IDF, 2001), using a nitrogen to protein conversion factor of 6.38.

For soluble protein analysis, denatured and aggregated protein material was removed by adjusting the sample to the isoelectric point at pH 4.6 using a 0.1 M acetate buffer to a final protein concentration of 2.5 g L^{-1} protein, centrifuging at 20,000 g for 20 min at 4°C and filtering through 0.2-µm low-protein binding PES filters (Agilent Technologies, California, United States). The prepared samples were evaluated using high-performance liquid chromatography (HPLC) using a Waters 2695 separation module, a Waters 2487 dual wavelength absorbance detector running on Waters Empower® software (Milford, MA, USA). Reversed-phase (RP) HPLC was completed using a PolymerX 5 µm RP-1, 150 x 4.6 mm column (Phenomenex, Cheshire, UK) as described by Kehoe *et al.* (2011). α-Lactalbumin, β-lactoglobulin A and β-lactoglobulin B standards (Sigma Aldrich, Ireland) were used to calibrate the method.

4.3.7. *Volatile analysis*

Volatile compounds were identified using head-space solid phase microextraction (HS-SPME) coupled with gas chromatography-mass spectrometry (GC-MS), described by Stefanovic *et al.* (2017), with some modifications. A number of temperatures were applied to induce volatile extraction ranging from 40 to 320 ºC and are identified in the original methodology. The sample volume was 4 mL and all samples were run in triplicate. Samples were processed using Shimadzu GCMS solutions software using the flavour and fragrance library (FFNSC 2) in combination with in house libraries and NIST 2011 Mass Spectral Library, AMDIS (www.amdis.net) software and linear retention indices were carried out using the method of Van den Dool and Kratz (1963). Batch processing was carried out with

metaMS (Wehrens *et al.*, 2014) (www.rdocumentation.org). The unheated and heat-treated dispersions were frozen, immediately after thermal processing, until required for volatile analysis.

4.3.8. *Statistical analysis*

All heat treatment trials were carried out in triplicate, and the subsequent data sets were subjected to analysis using the MINITAB® 15 (Minitab Ltd., Coventry, UK) statistical analysis package. The statistical significance of treatment effects on physical characteristics investigated was evaluated by means of one-way ANOVA with Tukey and Dunnetts' *post hoc* analysis. Three-way ANOVA was completed using the factors: protein content, heat treatment technology, and temperature of heat treatment and interactions between these factors. A paired *t*-test was carried out on particle size data to further investigate the effect of heat treatment. Principal component analysis (PCA) of protein beverage volatiles was performed using The Unscrambler X multivariate analysis programme, v10.3 (CAMO ASA, Trondheim, Norway).

4.4. Results

4.4.1. *Particle size and molecular weight distribution*

4.4.1.1. Particle size distribution

In general, the particle size (z-average) of the protein dispersions increased as a result of heat treatment (Table 4.1 and Table 4.2; $p < 0.001$). This was particularly the case in directly heated dispersions, with statistically significant increases found for directly ESL and UHT treated dispersions at 4 and 6 % (w/w) protein, and for directly ESL treated at 8% (w/w) protein, according to Dunnett's *post hoc* analysis data (not shown). A paired *t*-test revealed that indirect ESL heat treatments gave a higher particle size

than their indirect UHT-treated counterparts at 4, 6, and 8 % (w/w) protein concentrations ($p < 0.05$, 0.01 and 0.001, respectively), with the distinction between ESL and UHT treatments becoming stronger with increasing protein concentration. Directly heat-treated samples showed no significant difference in particle size between ESL and UHT treatments.

4.4.1.2. Molecular weight distribution

The MW profile of the aggregates formed in the soluble fraction of the beverage dispersions was determined using size-exclusion chromatography. The MW distributions were similar for the unheated dispersion at all protein concentrations, with high proportions of low MW proteins relative to native proteins (Fig. 4.2). For all heat-treated dispersions, the proportion of low MW aggregates decreased, while the presence of medium- and high-MW aggregates increased with increasing thermal load and protein concentration. For all protein concentrations, direct ESL treatment produced the lowest proportion of high MW aggregates (≥ 300 kDa) compared to all other heat treatments. In general, direct UHT, indirect ESL and indirect UHT

Table 4.1. Physicochemical properties of protein beverages containing 4, 6, or 8% total protein, before and after direct steam infusion and indirect tubular heat treatment[1].

Beverage Solutions	Heat Treatment	pH	Total Solids	Total Protein	Soluble Protein	Viscosity	Particle Size
		-	% (w/w)	% (w/w)	% (w/w)	mPa.s	Diameter (nm)
4% Protein	Unheated	$6.81^{a} \pm 0.03$	$4.13^{a} \pm 0.05$	$4.10^{a} \pm 0.08$	$3.57^{a} \pm 0.10$	$3.29^{ab} \pm 0.05$	$98.2^{c} \pm 0.76$
	Direct ESL	$6.84^{a} \pm 0.04$	$3.78^{b} \pm 0.06$	$3.82^{a} \pm 0.17$	$1.72^{b} \pm 0.29$	$3.33^{b} \pm 0.04$	$278^{a} \pm 2.42$
	Direct UHT	$6.91^{a} \pm 0.03$	$3.92^{ab} \pm 0.08$	$3.96^{a} \pm 0.01$	$1.20^{c} \pm 0.11$	$3.41^{ab} \pm 0.03$	$243^{ab} \pm 38.0$
	Indirect ESL	$6.89^{a} \pm 0.02$	$4.10^{a} \pm 0.08$	$4.08^{a} \pm 0.07$	$0.75^{c} \pm 0.14$	$3.49^{ab} \pm 0.02$	$218^{b} \pm 4.60$
	Indirect UHT	$6.92^{a} \pm 0.04$	$4.06^{a} \pm 0.07$	$4.08^{a} \pm 0.06$	$0.94^{c} \pm 0.06$	$3.53^{a} \pm 0.04$	$195^{b} \pm 17.2$
6% Protein	Unheated	$6.82^{ab} \pm 0.03$	$6.37^{a} \pm 0.08$	$6.18^{ab} \pm 0.05$	$5.85^{a} \pm 0.09$	$3.37^{b} \pm 0.03$	$121^{c} \pm 4.21$
	Direct ESL	$6.77^{b} \pm 0.02$	$5.96^{a} \pm 0.08$	$5.82^{bc} \pm 0.04$	$2.19^{b} \pm 0.18$	$3.42^{b} \pm 0.02$	$192^{ab} \pm 7.77$
	Direct UHT	$6.90^{a} \pm 0.07$	$5.82^{a} \pm 0.33$	$5.61^{c} \pm 0.04$	$1.36^{c} \pm 0.14$	$3.50^{b} \pm 0.07$	$168^{b} \pm 10.9$
	Indirect ESL	$6.85^{ab} \pm 0.02$	$6.29^{a} \pm 0.10$	$6.20^{a} \pm 0.13$	$0.75^{d} \pm 0.12$	$3.91^{a} \pm 0.02$	$216^{a} \pm 0.86$
	Indirect UHT	$6.87^{ab} \pm 0.02$	$6.25^{a} \pm 0.07$	$6.22^{a} \pm 0.14$	0.96 ± 0.08^{d}	$3.69^{ab} \pm 0.02$	$136^{c} \pm 12.5$
8% Protein	Unheated	$6.81^{a} \pm 0.04$	$8.44^{a} \pm 0.06$	$8.22^{a} \pm 0.07$	$7.71^{a} \pm 0.11$	$3.42^{d} \pm 0.04$	$97.4^{ab} \pm 1.48$
	Direct ESL	$6.81^{a} \pm 0.06$	$7.83^{c} \pm 0.16$	$7.56^{b} \pm 0.19$	$3.59^{b} \pm 1.22$	$4.10^{cd} \pm 0.06$	$244^{a} \pm 11.6$
	Direct UHT	$6.82^{a} \pm 0.07$	$8.02^{bc} \pm 0.12$	$7.86^{ab} \pm 0.08$	$1.30^{a} \pm 0.09$	$4.18^{bc} \pm 0.07$	$187^{ab} \pm 83.7$
	Indirect ESL	$6.83^{a} \pm 0.05$	$8.28^{ab} \pm 0.03$	$8.13^{a} \pm 0.03$	$0.67^{c} \pm 0.02$	$9.02^{a} \pm 0.05$	$211^{ab} \pm 4.57$
	Indirect UHT	$6.86^{a} \pm 0.01$	$8.39^{a} \pm 0.03$	$8.12^{a} \pm 0.06$	$1.00^{c} \pm 0.06$	$4.61^{a} \pm 0.01$	$114^{b} \pm 1.67$

1 For each beverage solution (protein concentration), mean values with a common superscript letter in the same column are not significantly different ($p > 0.05$). ESL relates to a 70°C preheat temperature and 121°C final heat temperature. UHT relates to an 80°C preheat temperature and 135°C final heat temperature.

Table 4.2. Statistical significance of the effects of target protein level, heating technology, severity of heat treatment and interactions of these factors on the physicochemical characteristics of heat treated solutions, assessed by three-way ANOVA[1].

Characteristic		Protein Level[2]	Technology	Heat Treatment	Protein Level[2]* Technology	Technology* Heat Treatment	Protein Level[2]* Heat Treatment
pH		**	NS	**	NS	NS	NS
Total Solids Content		***	***	NS	NS	NS	NS
Total Protein Content		***	***	NS	**	NS	NS
Total Soluble Protein Content		*	***	**	**	***	NS
Native Protein	α-La	NS	***	***	NS	***	NS
	β-lg A	*	***	***	NS	***	NS
	β-lg B	NS	***	***	NS	*	NS
Colour Coordinates	L*	***	***	***	***	*	***
	a*	***	***	***	***	*	*
	b*	*	***	NS	***	NS	*
Colour Difference, ΔE		***	***	***	***	***	***
Viscosity		***	***	***	***	***	***
Particle Size		***	***	***	NS	NS	NS
Molecular Weight Distribution	≥ 300 kDa	***	***	***	**	***	NS
	80-300 kDa	***	NS	NS	***	NS	NS
	30-80 kDa	***	NS	*	**	NS	NS
	15-30 kDa	***	***	***	NS	***	NS
	8-15 kDa	***	***	***	NS	***	NS

[1] *** indicates $p<0.001$, ** indicates $p<0.01$, * indicates $p<0.05$ and NS indicates no significant difference.
[2] Protein level refers to the target protein content to which the solutions are formulated.

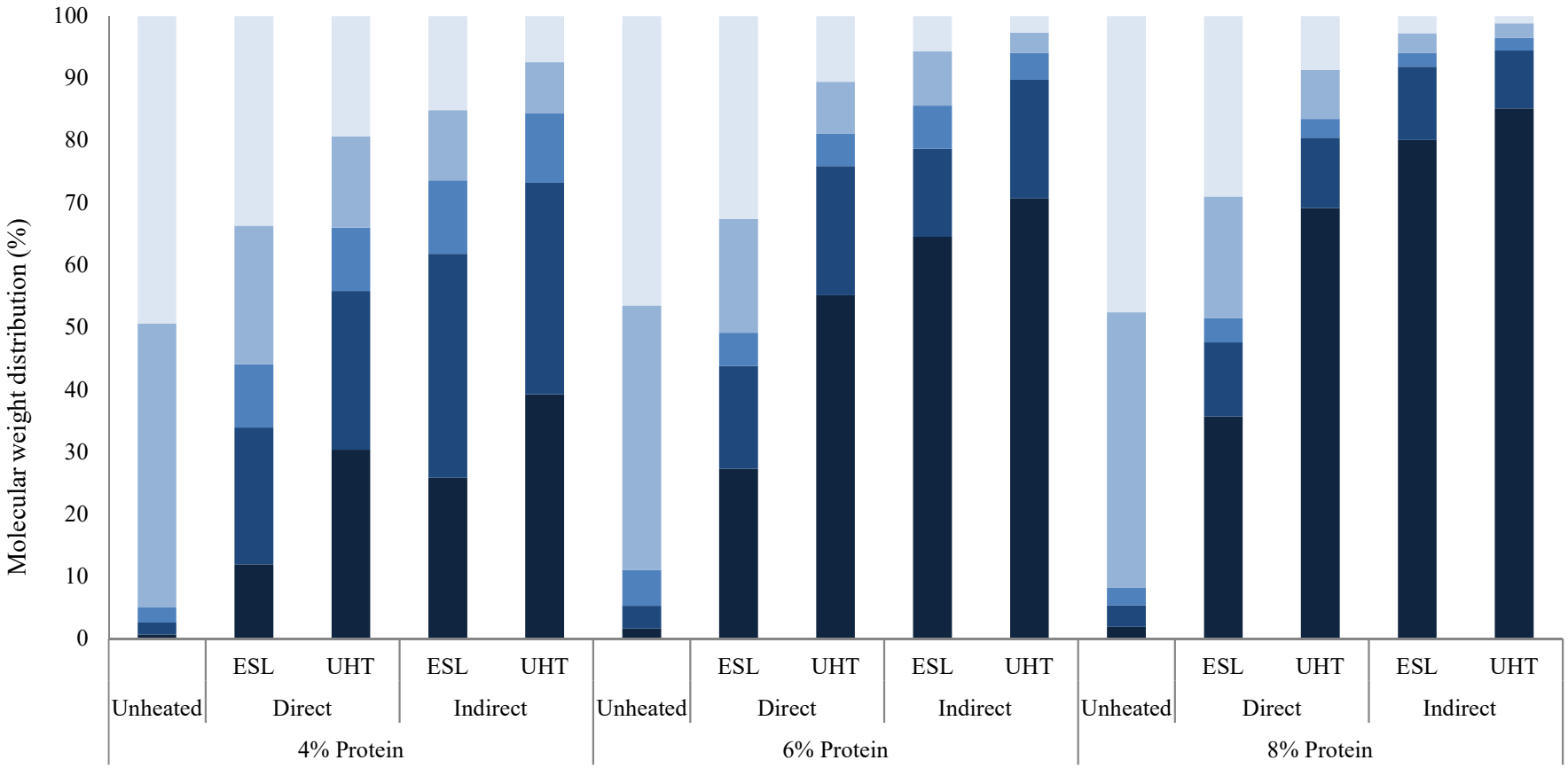


Fig. 4.2. Molecular weight distribution of the soluble fraction of unheated and heat-treated whey protein dispersions with molecular weights of 8-15 kDa (□), 15-30 kDa (□), 30-80 kDa (□), 80-300 kDa (□), 300+ kDa (□).

treatments resulted in statistically similar MW profiles for the soluble phase. The difference in the proportion of particles with a MW greater than 300 kDa between direct and indirect UHT treatments increased with increasing protein concentration, resulting in a significantly greater proportion of high MW aggregates in the soluble fraction following indirect UHT treatment for 8% (w/w) protein concentration compared to those which were directly treated.

The proportion of total protein material with a MW of 8-15 kDa decreased significantly for all heat treatments except for the direct ESL treatment at 4% protein. The proportion of protein material with a MW of 8-15 kDa were not significantly different between direct UHT, indirect ESL and indirect UHT in most cases, although the proportion could be seen to decrease as the thermal load increased, i.e., direct UHT > indirect ESL > indirect UHT.

4.4.2. *Colour analysis*

All heat treatments resulted in a significant change in L* value or lightness, from the unheated dispersion, with the exception of ESL and UHT indirectly treated 8% (w/w) dispersion (Table 4.3). The protein content of dispersions, heating technology and heating temperature each had a significant effect on L* ($p < 0.001$; Table 4.3 and Fig. 4.3). For 4% protein dispersions, the lightness was similar for direct and indirect UHT heat treatments, while the corresponding direct and indirect ESL-treated dispersions were statistically different from each other. Direct ESL heat treatment at 6% (w/w) protein resulted in a significantly higher L* value than all other heat treatments for 6% (w/w) protein. Indirect UHT treatment resulted in a significantly lower L* value compared to that of all other heat treatments at 6% protein. For 8% protein dispersions, the L* of both direct heat treatments was significantly greater than after indirect heat treatments. A paired *t*-test showed that dispersions treated by indirect ESL had a higher

L* value than their indirectly UHT-treated counterparts ($p < 0.01$). Similar to the L* value, the a* value was significantly reduced by heat treatment, implying a reduction in redness, with the exception of indirect heat treatments at 8% (w/w) protein concentration. Heat treatment significantly reduced the b* value of all protein concentrations, implying a reduction in measured yellowness (Table 4.3). These changes in colour identified are visually observable and may have an impact on consumer perception.

Table 4.3. Whey protein beverage colour, expressed as L*, a*, b* values for protein beverages containing 4%, 6%, or 8% total protein, before and after direct steam infusion and indirect tubular heat treatment[1].

Solutions	Heat Treatment	L*	a*	b*
4% Protein	Unheated	$39.3^{c} \pm 1.21$	$-0.65^{a} \pm 0.09$	$2.38^{a} \pm 0.35$
	Direct ESL	$64.2^{b} \pm 1.35$	$-1.46^{b} \pm 0.29$	$-5.14^{b} \pm 0.85$
	Direct UHT	$66.3^{ab} \pm 1.92$	$-1.85^{b} \pm 0.12$	$-5.27^{b} \pm 0.45$
	Indirect ESL	$68.8^{a} \pm 0.92$	$-2.30^{c} \pm 0.01$	$-6.60^{b} \pm 0.23$
	Indirect UHT	$66.5^{ab} \pm 0.80$	$-2.34^{c} \pm 0.02$	$-8.33^{c} \pm 0.47$
6% Protein	Unheated	$32.6^{d} \pm 0.82$	$-0.13^{a} \pm 0.03$	$0.76^{a} \pm 0.42$
	Direct ESL	$67.8^{a} \pm 1.30$	$-1.82^{cd} \pm 0.18$	$-5.15^{b} \pm 1.09$
	Direct UHT	$63.7^{b} \pm 2.02$	$-1.47^{c} \pm 0.23$	$-4.27^{b} \pm 0.70$
	Indirect ESL	$60.2^{b} \pm 0.77$	$-2.02^{d} \pm 0.02$	$-8.45^{c} \pm 0.21$
	Indirect UHT	$46.7^{c} \pm 0.22$	$-0.73^{b} \pm 0.04$	$-10.9^{d} \pm 0.09$
8% Protein	Unheated	$36.6^{b} \pm 0.41$	$-0.23^{a} \pm 0.07$	$2.81^{a} \pm 0.24$
	Direct ESL	$60.2^{a} \pm 1.86$	$-1.79^{b} \pm 0.11$	$-6.83^{c} \pm 0.74$
	Direct UHT	$63.6^{a} \pm 3.85$	$-1.69^{b} \pm 0.45$	$-3.09^{b} \pm 1.57$
	Indirect ESL	$41.5^{b} \pm 0.71$	$-0.32^{a} \pm 0.19$	$-7.21^{c} \pm 0.49$
	Indirect UHT	$38.1^{b} \pm 0.37$	$0.35^{a} \pm 0.08$	$-6.20^{c} \pm 0.26$

[1] For each beverage solution (protein concentration), mean values with a common superscript letter in the same column are not significantly different ($p > 0.05$). ESL relates to a 70°C preheat temperature and 121°C final heat temperature. UHT relates to 80°C preheat temperature and 135°C final heat temperature.

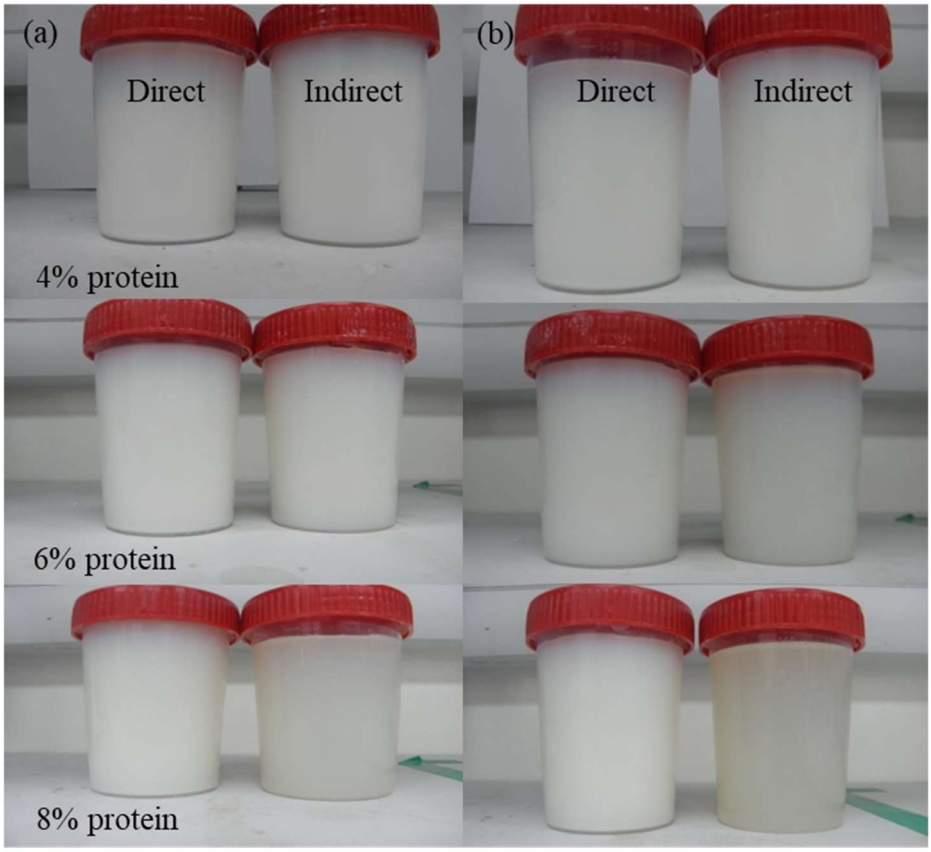


Fig. 4.3. Images of whey protein dispersions at 4, 6 and 8% (w/w) protein after direct and indirect with (a) ESL (70 °C preheat and 121 °C) and (b) UHT (80 °C preheat and 135 °C) heat-treated formulations.

4.4.3. *Viscosity*

Protein concentration, choice of heating technology and severity of heat treatment all had a significant effect on the viscosity of protein dispersions as determined by three-way ANOVA ($p < 0.001$; Table 4.2). The extent of increase in viscosity upon heating increased with increasing protein concentration of the dispersions, where the 8% (w/w) protein dispersions were the most affected by heat treatment. Overall, direct heat treatment resulted in a lower final viscosity than indirect heat treatment, although this difference was not statistically significant in some cases below 8% protein level.

While 4% (w/w) protein dispersions showed no significant viscosity increase on heating, the viscosity of indirectly-treated 6% (w/w) protein dispersions increased

significantly with ESL treatment. At 8% (w/w) protein, heat-treated dispersions showed a significant increase in viscosity during heat treatment, with direct ESL and UHT treatments resulting in similar viscosities, which were lower than that achieved by indirect heating. Similar to the trends for 6% (w/w) protein dispersions, indirect ESL treatment of 8% (w/w) protein dispersions resulted in a significantly higher viscosity (9.02 mPa.s) compared to indirect UHT treatment (4.61 mPa.s), despite the higher final heating temperature in the latter. For indirect heating, there was a statistically significant interaction determined between the heating technology and heat treatment temperature ($p < 0.001$).

4.4.4. *Protein content, profile and level of soluble protein*

4.4.4.1. Total solids and protein content of WPI dispersions

Direct heating was associated with significantly decreased total solids contents of dispersions, in some cases with reductions of 4.95 – 8.58 %, and the effect was particularly significant around 8 % protein level (Table 4.1), while the total solids content was unaffected by indirect heat treatment for all protein concentrations. Three-way ANOVA analysis confirmed that heating technology had a significant effect reducing the total solids level ($p < 0.001$), while the severity of heat treatment (i.e., ESL or UHT) did not affect total solids content (Table 4.2).

The total protein content of unheated and heated dispersions followed similar trends to that of total solids due to the high protein content of the WPI powder used in dispersions (Table 4.1 and 4.2). While reductions in total protein content were observed for all directly heated dispersions, this reduction was only statistically significant for dispersions containing 6 and 8% (w/w) total protein. The reduction in total solids and total protein observed in directly heat-treated dispersions (i.e. steam

injection and infusion) is likely the result of dilution, with condensed steam not being completely removed by flash cooling during direct processing. Product dilution, or concentration, during direct heating is common, and has been reported in numerous studies (Lewis *et al.*, 2000; Murphy, 2011; Dickow *et al.*, 2012a; Murphy *et al.*, 2013; Dumpler *et al.*, 2017). Net dilution or concentration within the system can be reduced by maintaining equal temperatures at preheat and flash cooling stages and implementing finer instrument control.

4.4.4.2. Soluble protein

RP-HPLC showed that direct and indirect heat treatment resulted in significant levels of whey protein denaturation compared to the unheated dispersions (Fig. 4.4). Three-way ANOVA analysis of RP-HPLC data revealed that all protein fractions investigated were significantly affected by heating technology ($p < 0.001$) and the temperature of heat treatment ($p < 0.001$). Direct heating resulted in lower levels of protein denaturation (i.e. more native protein) for direct ESL thermal treatment in particular. Direct ESL heat treatments resulted in the retention of significantly high levels of native α-lactalbumin (α-la) compared to indirect heating, for all dispersions tested ($p < 0.05$). The lowest level of native α-la was obtained using indirect UHT treatment, to a significant degree for the 4 and 6% (w/w) protein dispersions ($p < 0.05$). Although directly UHT-treated dispersions had a higher level of native α-la after heat treatment than indirect ESL treatment, the difference was not statistically significant in most cases (Table 4.1). For both the β-lactoglobulin A (β-lg A) and B (β-lg B), direct ESL treatment resulted in the lowest levels of denaturation, with the exception of the level of β-lg A in the 6% protein dispersion which, while lower, was not statistically different from that of the other heat treatments.

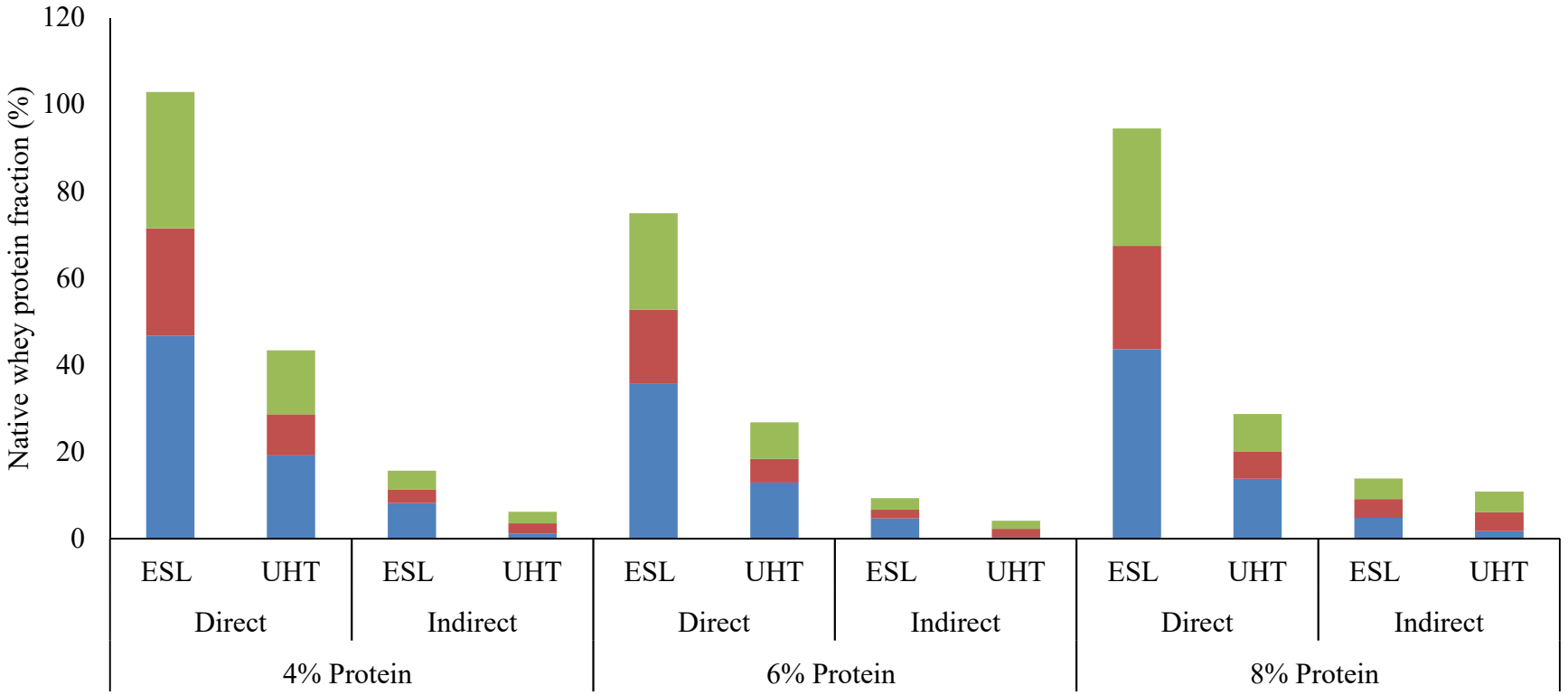


Fig. 4.4. Levels of native whey protein in the pH 4.6-soluble fraction measured by RP-HPLC; α-lactalbumin (■), β-lactogloblin B (■), and β-lactoglobulin A (■) expressed as a percentage of total native whey protein for whey protein beverage dispersions at 4%, 6%, and 8% (w/w) total protein.

4.4.5. *Volatile analysis*

A range of 62 volatile aromatic organic compounds were identified in the beverage dispersions, including ketones, aldehydes, alcohols, esters, furans, sulphur- and benzene-containing compounds (results not shown). Differences between directly and indirectly treated dispersions were identified for many compounds. Indirect treatment increased levels of aldehyde compounds were observed ($p < 0.05$), such as pentanal, hexanal, heptanal, octanal and 2-methylpropanal, which is known to promote the 'stale' flavour in high-temperature-treated milks (Zabbia *et al.*, 2012). A significant increase in the levels of dimethyl trisulphide and other sulphur compounds was found for indirectly heat-treated dispersions ($p < 0.05$). Such sulphur compounds are related to strong 'cooked' flavours in high temperature treated milks as a result of β-lactoglobulin denaturation (Al-Attabi *et al.*, 2008). The generation of furan compounds was also noted, although the increased levels of 2-pentylfuran and 2-butylfuran with indirect heating were not significantly higher than those following direct heating.

The PCA plot shows that the volatiles profile of heat treated dispersions can be discriminated on the basis of the heating technology and severity of thermal treatment applied, particularly for indirect heat treatment (Fig. 4.5). The volatile profile of directly-heated dispersions related more closely to unheated dispersions than to those which were indirectly-heated. Although some differences between unheated and direct ESL dispersions could be observed, particularly for the 8% (w/w) protein dispersion, as protein concentration increased, direct ESL treatment and unheated controls were shown to have a strong PCA grouping indicating relative similarity between the volatile profiles of the treatments.

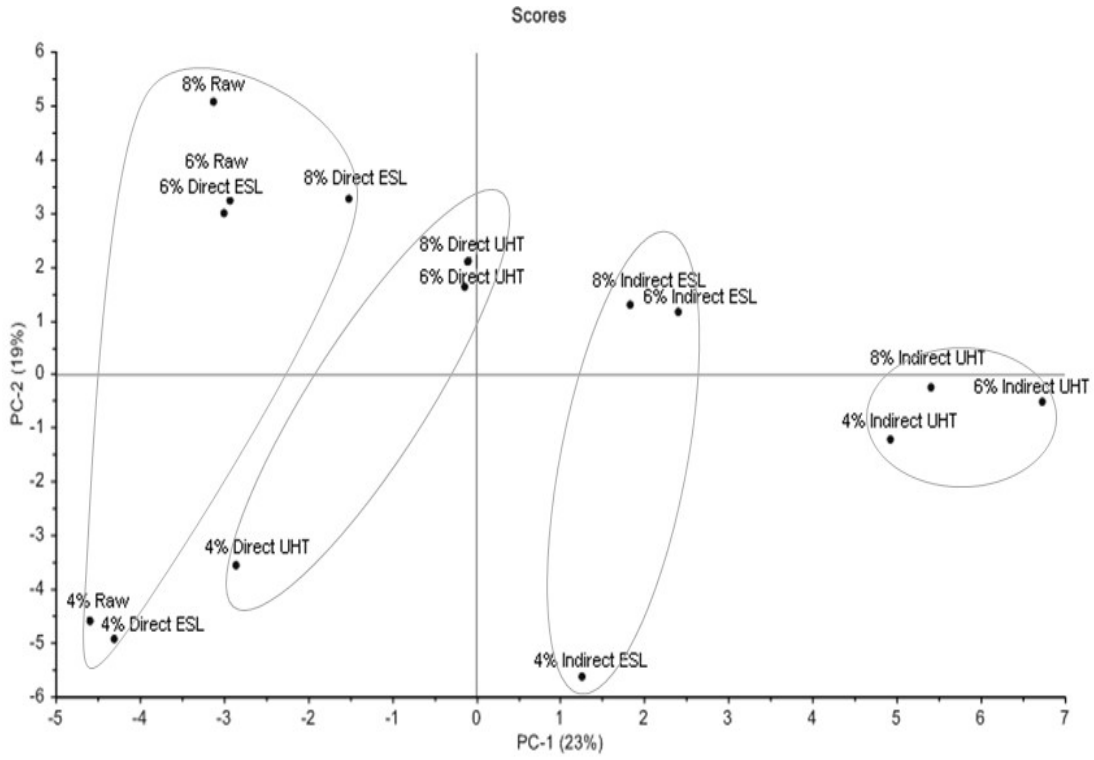


Fig. 4.5. Principal component analysis plot of the volatile profiles of unheated, directly and indirectly heated whey protein dispersions with 4%, 6%, or 8% total protein.

More distinctive grouping was observed for the direct UHT treated dispersions. However, indirect heat treatment of dispersions resulted in clear differences between the unheated, ESL and UHT dispersions, which increased as the heating temperature increased. The PCA plot also showed differences based on protein content, which may have been due to a higher level of *d*-Limonene found in 4% (w/w) protein dispersions than in higher protein content dispersions, although the difference levels were not statistically significant. *d*-Limonene is a terpene derived from animal feed and commonly found in milk; levels will vary dependent upon diet and metabolism in the rumen (Hansen and Heinis, 1992).

4.5. Discussion

The application of direct and indirect heating technologies resulted in significant differences in the physical characteristics of the high protein dispersions. These differences have the potential to impact consumer perception and acceptability, as they relate to protein bioavailability, appearance and volatile profile of the final product.

A significantly higher level of soluble protein was recorded following direct heat treatment compared to indirect heat treatment. This reduced level of protein denaturation can be attributed to the lower overall thermal load imparted due to rapid heating and cooling (Fig. 4.1c) (Burton, 1994; Lewis *et al.*, 2000; Murphy *et al.*, 2013). Pellegrino (2013) reported that the retention of a higher level of native whey proteins preserves the nutritional quality and digestibility of proteins in dispersions which may be of interest to health-conscious consumers of high protein beverages. Direct ESL treatment resulted in less protein denaturation for all dispersions, and the level of protein denaturation increased (albeit not to a significant degree in all cases) as the thermal load increased, i.e., Direct ESL < Direct UHT < Indirect ESL < Indirect UHT. These ranges are consistent with those reported in previous studies (Burton, 1994; Lewis *et al.*, 2000; Elliott *et al.*, 2003).

The appearance of directly and indirectly treated dispersions was noticeably different. While directly-treated dispersions were equally opaque at each of the protein concentrations, indirectly-treated dispersions were seen to have reduced opacity as the protein concentration increased, as measured by a reduction in L* value (Fig. 4.3 and Table 4.3). The significant changes in L* were consistent with some general trends in particle size. For indirectly-treated dispersions, ESL-treated dispersions had a greater particle size and L* value than their UHT-treated counterparts, as predicted by

Rayleigh's Law, which relates particle size to colour change (Desobry-Banon *et al.*, 1994; McClements, 2002; Chung *et al.*, 2014). This increased level of whiteness in whey protein dispersions obtained from direct heating systems may have a knock-on impact on customer perception.

Some directly-treated dispersions were found to have a larger particle size compared to indirectly-treated dispersions, despite having a lower degree of whey protein denaturation. These findings may seem counterintuitive; however, this is in agreement with the findings of previous studies (Burton, 1968; Datta *et al.*, 2002; Malmgren *et al.*, 2017) that proposed that the presence of some larger aggregates was related to reduced levels of deposition and fouling in direct heating systems. As the larger aggregates are not retained on heat transfer interfaces within the heating system during direct steam infusion, they remain in the product stream, contributing to increased whiteness and particle size. The difference in particle size may also be related to differences in denaturation and aggregation mechanisms due to the thermal profiles of the direct and indirect systems (Fig. 4.1c). Denaturation and aggregation occur in two distinct stages; the first consists of the unfolding of β-lg, and the second involves the association of these unfolded molecules to form aggregates (Mulvihill and Donovan, 1987; Joyce *et al.*, 2017). Anema *et al.* (1996) found that aggregation of unfolded proteins was the rate-determining step during high-temperature processing of directly heat-treated reconstituted whole milk. The different thermal profile of the two thermal processing technologies could lead to the formation of different types of aggregates after denaturation as a result of these mechanisms.

As the average particle size of indirectly treated dispersions decreased, the viscosity of the dispersions increased, due to an increase in particle-particle interactions between a larger number of smaller particles (Table 4.1). Indirect ESL treatment

resulted in a large increase in viscosity, from 3.42 to 9.02 mPa.s, compared to both direct heat treatments and to the indirect UHT treatment, despite the higher final heating temperature. This may be due to the effect of preheating temperature, which has been shown to impact the heat stability of protein dispersions, stabilising against heat-induced physical changes during high temperature processing (Srichantra, 2006; Drapala *et al.*, 2016; Dumpler and Kulozik, 2016). In this study, no such effect was seen when direct heat treatment was applied, suggesting that preheat treatment may have a less significant effect during direct heating compared to indirect.

Jansson *et al.* (2014) reported that the severity of heat treatments related to the development of off-flavours in milk. The results of the present study are consistent with this, as direct heat treatment, with its lower thermal load, produced a volatile profile which was closer to that of the unheated dispersion than its indirect counterpart. In addition to the reduced severity of heating during direct heat treatment, studies have shown that the rapid vacuum flash cooling step in this process can also aid in the removal of volatiles, improving the flavour of heat-treated dispersions (Deeth and Lewis, 2016; Lee *et al.*, 2017).

4.6. Conclusion

The application of direct or indirect heating technology had a significant impact on the end-product functionality, appearance and sensory properties of whey protein dispersions. Direct heating resulted in many favourable product properties and significantly less thermal damage across all protein concentrations compared to indirect heating. This direct heating technology enabled the retention of higher levels of native whey protein, as determined by RP- and SE-HPLC, lower viscosity and minimal change in volatile profile. However, the products produced were more opaque

than indirectly heat-treated dispersions, particularly at higher protein concentrations. Direct heat treatment can be used to process challenging whey protein beverages with a high-protein content, achieving final product properties that are unattainable with traditional indirect heat treatment methods. The application of this technology to the growing high-protein beverage market would result in products with greater nutritional value and flavour.

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Chapter 5.

Development of a pilot-scale direct supersonic steam injection system and commissioning with skim milk

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5.1. Abstract

Supersonic steam injection heating (SSIH) technology is a novel iteration of traditional direct steam injection, the application of which has not been extensively researched in relation to dairy processing. A pilot-scale SSIH line, incorporating a patented supersonic injector, was developed and commissioned using skim milk across a two preheat (70 and 85 °C) and four final heat temperatures (121, 128, 135, 142 °C). As experimental controls, the above temperature combinations were also applied using an indirect (IND) tubular heat exchanger-based pilot plant, and the physical characteristics of the products from both heating technologies were compared. SSIH technology resulted in significantly less protein denaturation compared to IND-treated milks. However, characteristics like average particle size, apparent viscosity, and colour difference were higher for SSIH-treated milks overall. While SSIH technology could be used to produce a milk with significantly more native whey protein, there may be a trade off in terms of the physical stability of the product.

5.2. Introduction

Milk, as a commercial food product, is required to undergo heat treatment to reduce microbial load to ensure safety for commercial consumption (Lewis and Deeth, 2009). There are numerous heat treatments of varying severity which result in shelf lives ranging from 3 days to 2 years. These treatments include thermisation, pasteurisation, extended shelf-life (ESL) and ultra-high-temperature (UHT) processing and in-container sterilisation. The most common treatments among those are high-temperature short-time (HTST) pasteurisation (72 °C for 15s) giving a refrigerated shelf life of 2 to 3 weeks, and ESL or UHT treatments (125-138 °C or 135-150 °C for 2-4 secs, respectively) which give a longer shelf life, from several months up to 2 years without cold chain storage requirements (Fox *et al.*, 2015, Lee *et al.*, 2017). Improving product shelf-life and eliminating cold chain requirements reduces the economic costs of producers, distributors and subsequently consumers, particularly in areas where long-distances and refrigeration limitations make storage problematic (Bylund, 1995, Rysstad and Kolstad, 2006, Saravacos and Kostaropoulos, 2016). While longer life milks, such as ESL and UHT milks, have gained general acceptance, they are still rejected in many regions due to a perceived 'cooked' flavour reported by consumers (Al-Attabi *et al.*, 2008, Jansson *et al.*, 2014, Malmgren *et al.*, 2017). These forms of severe heat treatment may also result differences to product physical properties such as increased protein denaturation, colour change and sedimentation compared to pasteurised milk, affecting the physical stability of the final product and general consumer acceptance (Chen *et al.*, 2015, Deeth and Lewis, 2016).

The heating technology used for processing can have a significant effect on the physical properties of a product subjected to severe heat treatment. Heating technologies can be divided into two general categories; direct and indirect (Lewis *et*

al., 2000). Indirect heating, commonly used in the food industry, achieves heat transfer through a soild medium separating the product from the heating medium as seen in plate, tubular, and scraped surface heat exchangers, while direct heating involves steam being mixed into the product by direct infusion or injection systems and being subsequently removed by vacuum cooling. Direct heating is a more rapid and making use of the latent heat of evaporation as steam condenses, and imparting a reduced thermal load compared to indirect heat treatment (Britz and Robinson, 2008, Saravacos and Kostaropoulos, 2016).

Traditional direct and indirect technologies have been available for decades and their use with various dairy products has been investigated throughout the years (Lyster *et al.*, 1971, Datta *et al.*, 2002, Dumpler and Kulozik, 2016). Over the years there have been engineering improvements and reconfigurations made to the original formats, such as the lenient steam injection patented process (Dickow *et al.*, 2012a, Dickow *et al.*, 2012b) or supersonic steam injection (Murphy, 2011, Murphy *et al.*, 2013). There has been little investigation into the application and potential benefits of supersonic steam injection to dairy products.

Supersonic flow can be achieved using a De Laval nozzle which utilises changes in the nozzle area and the conservation of volumetric flow to transform a subsonic flow into a supersonic flow. This nozzle was first developed in 1890 by Gustaf de Laval for use on a steam turbine and is commonly used today in rocket and jet engines (Johnson, 2016). These nozzles are commonly referred to as converging-diverging nozzles and consist of three regions: an inlet converging into a narrow throat and subsequently expanding into a divergent outlet (Canosa *et al.*, 2016). The converging inlet accelerates the fluid velocity, which assists the mixing with steam in an efficient manner; this acceleration can continue until the flow becomes choked or sonic at the

throat, where the cross-sectional areas is smallest, and a Mach number (M) of 1 is achieved. Temperature and pressure reduce in the diverging outlet with increasing kinetic energy resulting in supersonic flow (M > 1). The application of a supersonic steam injector could theoretically produce better mixing due to the shockwave produced and reduced residence time due to the supersonic flow achieved compared to traditional steam injections units in dairy processing.

The aim of this study was to develop a pilot-scale supersonic injection system, commission the system using skim milk and investigate the effect of indirect tubular and direct supersonic injection heating (SSIH) technology on the physico-chemical properties of skim milk.

5.3. Materials and Methods

5.3.1. *Materials*

Skim milk was obtained from Dairygold Co-operative Society Ltd. (Clonmel Road, Mitchelstown, Co. Cork). The milk composition was 3.45% protein, 0.05% fat, 4.84% lactose and 8.92% total solids.

5.3.2. *Pilot-scale plant design*

To investigate the application of supersonic steam injection heating (SSIH), a purpose-built process line integrating a patented supersonic steam injector (Maklad Innovative Fluid- and Systemtechnik GmbH, Austria) was designed. Fabrication of the SSIH line was completed by Complete Stainless Engineering Ltd. (Limerick, Ireland). The SSIH line consisted of the SSIH injector, flash cooler, condenser, product pumps, culinary steam and product filters and an independent CIP system (Fig. 5.1).

Fig. 5.1. SSIH process line where the main components of the line are shown; (1) steam supply and culinary steam filter, (2) SSIH injector, (3) holding tube, (4) flash vessel and condenser, (5) product return pump, (6) CIP tank, (7) control panel, and (8) vacuum pump.

This line was connected to an existing indirect tubular UHT 25HV pilot plant (Microthermics, NC, USA), utilising the tubular heat exchangers for preheating and final cooling operations (Fig. 5.2). The system can be set up for an indirect (IND) tubular or direct SSIH final heat operation with common preheat and cooling tubular heat exchanger systems. This plant design enables more accurate comparison of the effect of SSIH and IND technologies on final product quality.

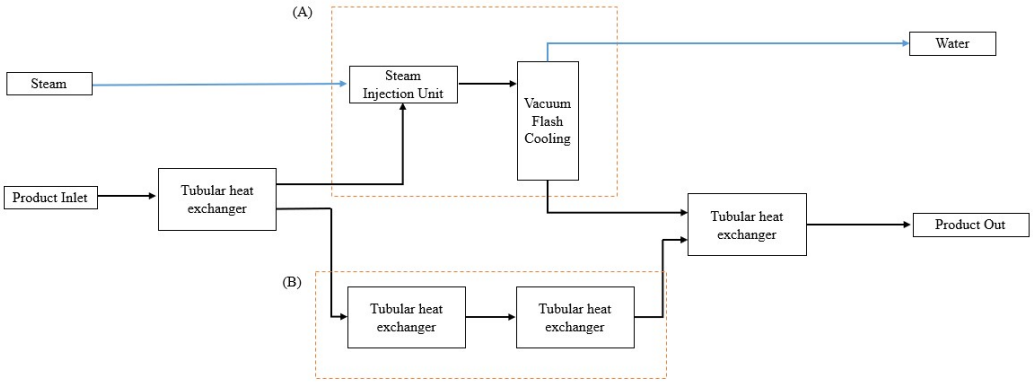

Fig. 5.2. Process flow diagram of the (A) direct steam injection (SSIH) and (B) indirect tubular (IND) heating pilot scale plants with common preheat and final cooling tubular heat exchangers between both plants

The SSIH injector had a flowrate capability of 50 – 150 L/hr, aligning with the capacity of the IND system and an available steam pressure of 6 bar(a). The injector can potentially achieve supersonic flow through the utilisation of a converging-diverging *de Laval* nozzle (Fig. 5.3). The nozzle design also features a Teflon coating in the steam-product mixing zone to reduce burn-on.

A simulator was developed in Microsft Excel to predict steam requirements and processing considerations for any scale of production, product flow and energy based on the processing settings and equipment details input into the program. This simulator can be used for scaling direct heating systems and for planning processing trials. The design of the injector was also evaluated to analyse the thermodynamic conditions and steam requirements under the experimental conditions. These pilot-scale injector and plant design considerations are described in Section 5.4.

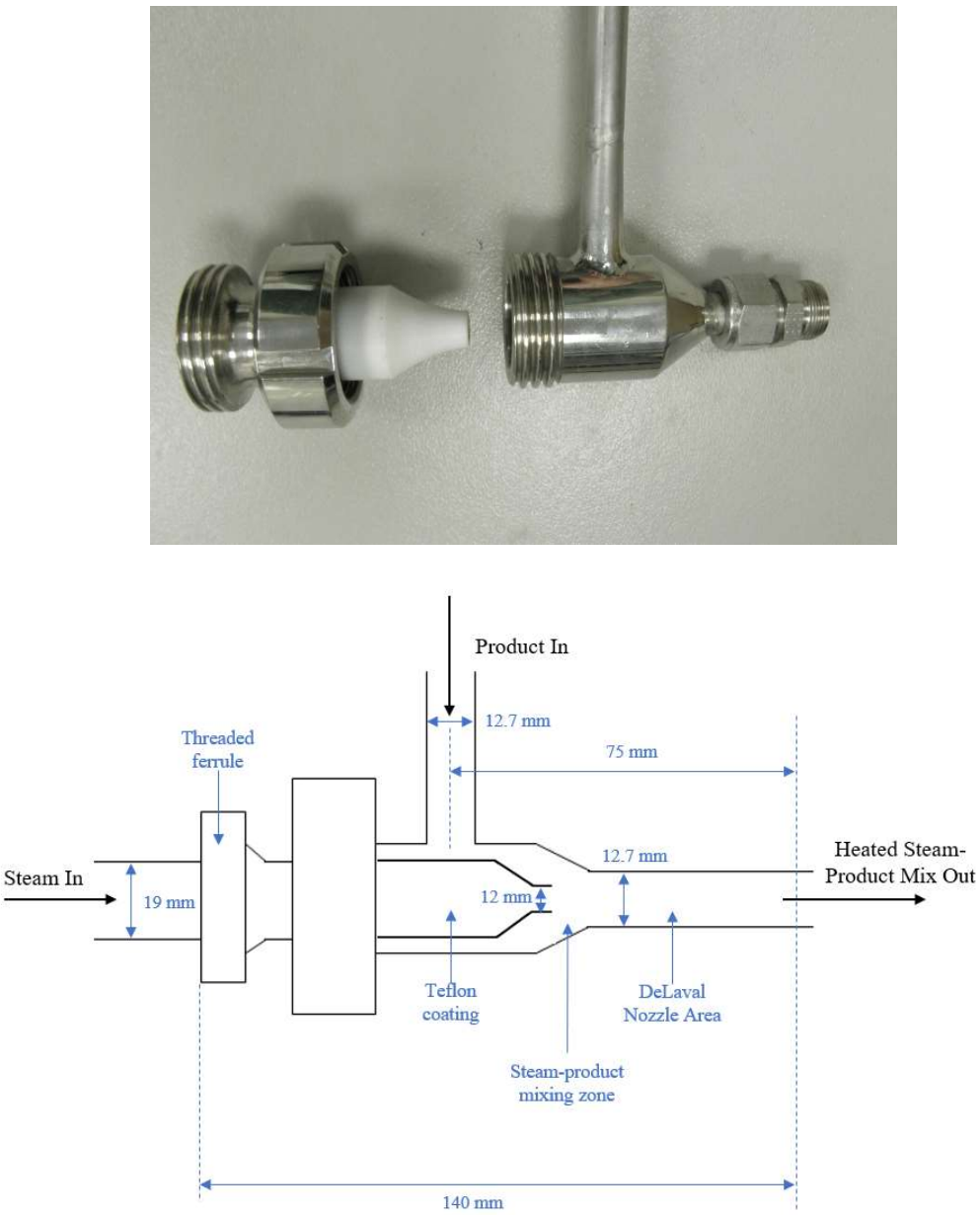

Fig. 5.3. (a) Image of the supersonic steam injection unit and (b) associated dimensions

5.3.3. *Heat treatment*

A series of trials was carried out on skim milk using direct steam injection (SSIH) heating and indirect (IND) tubular heating with common tubular preheat and final cooling heat exchangers. The experimental design consisted of heat treatment combinations using both SSIH and IND heating technologies with preheat temperatures of 70 and 85 °C for 30s, combined with final heat temperatures of 121,

128, 135 and 142 °C held for 2s with a flow rate of 100 L/hr. The residence time of the tubular heating, steam injection and flash cooling operations were taken as 30, 1 and 1s for 100L/hr. These trials were carried out in duplicate. Despite the application of common holding tube temperature-time combinations, SSIH applies a reduced thermal load to the product due to rapid heating and cooling processes, and reduced residence time compared to IND.

A calculation of the F_0 value, a bacterial lethality index, was completed for the two heat treatment technologies to ensure the differences in thermal load, due to heating and cooling times, did not significantly impact the predicted decimal reduction of bacterial organisms and comparability of the systems. The F_0 was determined using the following equation:

$$F = \int_0^{\infty} 10^{(\theta-\theta_{ref})/z} . \mathrm{dt} \qquad 5.1$$

Where the reference temperature, θ_{ref}, is 121.1 ºC and the z-value is 10 ºC as determined for *Clostridium botulinum* spores (Lewis and Heppell, 2000). It should be noted that across the heat treatment temperatures applied the F_0 values varied slightly between technologies with ranges of 0.49 – 0.54 for 121ºC final heat, 2.44 – 2.64 for 128ºC final heat, 12.25 – 12.95 for 135ºC final heat and 61.37 – 63.96 for 142ºC final heat with 70 and 85ºC preheat temperatures. The difference in the calculated F_o value increased with increasing final heat temperature.

5.3.4. *Total solids analysis*

Total solids content of milk was measured using a Smart System 5, Smart Trac (CEM Corporation, Matthews, NC, USA).

5.3.5. *Particle size distribution*

Particle size distribution of colloidal proteins in the skim milk samples was determined using dynamic light scattering with a Malvern Zetasizer Nano Zs instrument (Malvern Instruments Ltd., UK). Skim milk samples were dispersed in distilled water for analysis in polystyrene disposable cuvettes. Refractive indeces of 1.45 and 1.33 were assigned for protein and dispersant, respectively. All samples were analysed at a temperature of 25 °C.

5.3.6. *Viscosity*

Viscosity was measured using an AR G2 rheometer (TA Instruments, Crawley, UK) equipped with concentric cylinder geometry at 25 °C. The procedure involved the samples being pre-sheared at 500 s^{-1} for 1 min followed by equilibration at 0 s^{-1} for 1 min. The shear rate was then increased from 5 to 500 s^{-1} over 2 min, held at 500 s^{-1} for 1 min then decreased from 500 to 5 s^{-1} over 2 min. Viscosity reported is the average viscosity measured during the holding stage at 500 s^{-1} (Murphy *et al.*, 2013).

5.3.7. *Whey protein denaturation*

Reverse-phase high-performance liquid chromatography (RP-HPLC) was carried out to observe the loss in native protein structure, using a Waters 2695 separation module, a Waters 2487 dual wavelength absorbance detector running on Waters Empower® software (Milford, MA, USA) and a Source^{TM} 5RPC, 150 mm x 4.6 mm column (GE Healthcare, Buckinghamshire, UK). Soluble protein solutions (20 µL, 2.5 g L^{-1}) were loaded onto the column which was equilibrated at 28 °C and the column eluate was monitored at a detector wavelength of 214 nm (Kehoe *et al.*, 2011).

Proteins which possess the same retention time as procured unheated standards under gradient elution were designated ‘native’, these whey protein standards were α-

lactalbumin, β-lactoglobulin, and bovine serum albumin (BSA) (Sigma-Aldrich). The level of native protein for each whey protein was determined and expressed as a percentage of the initial content in the unheated control skim milk.

5.3.8. *Colour analysis*

The colour of each solution was measured and expressed as L*, a* and b* values using a Minolta Chroma Meter CR-400 colorimeter (Minolta Ltd., Milton Keynes, UK). The L* value indicates lightness, a* values indicate redness-greenness, b* values indicate yellowness- blueness. Samples were loaded into a disposable cuvette and places in front of a white calibration plate (L*, a*, b*) before measurement in triplicate.

Colour difference, ΔE, was used as a metric in determining the colour change from unheated skim milk. To get a basic reading for colour difference the CIE76 formula was used:

$$\Delta E = \sqrt{(L_2^* - L_1^*)^2 + (a_2^* - a_1^*)^2 + (b_2^* - b_1^*)^2} \qquad 5.2$$

5.3.9. *Accelerated physical stability*

The stability of the product was determined as described in Murphy *et al.* (2011). A Lumifuge 116 stability analyser (L.U.M. GmbH, Berlin, Germany) was used to measure the separation rate of samples (0.4 mL aliquots at 1500 rpm for 7.2 hr), determined using SepView 4.1 software (L.U.M. GmbH, Berlin, Germany) and reported as percentage transmission/second. The software uses integration with respect to position on each transmission profile to characterise instability over time as a second order polynomial. The average slope of this polynomial, calculated from the polynomial coefficients, was used as an instability index to compare the stability of different samples.

5.3.10. *Statistical analysis*

Statistical analysis was carried out using Minitab® 17 (Minitab Ltd., Coventry, UK) statistical analysis package. One-way analysis of variance (ANOVA) with Tukey *post hoc* analysis was applied to identify significant differences in treatment means at $p < 0.05$. In addition, three-way ANOVA was undertaken to determine statistical significance and effects of specific factors: type of technology, preheat temperature, and final heat temperature, and interactions between these factors.

5.4. Calculations

The ratio of steam to product, on a water basis, required to achieve the desired increase in product temperature, ΔT, can be calculated as:

$$z = \frac{c_p \Delta T}{h_1 - h_f} \quad 5.3$$

where h_1 is the specific enthalpy of the steam at the nozzle inlet, h_f is the specific enthalpy of the product at the final temperature and c_p is the specific heat of the product; the value of c_p is made up of contributions from water (the major component) and also of fat and non-fat solids. If the incoming product is preheated, to T_i = 70 °C, with a typical final heat temperature, T_f = 121 °C, after mixing with steam at 3 bar(a), the ratio of condensed steam to incoming product to achieve the desired product temperature (121°C) is 9.5% by weight.

The mass flowrate of the heated steam-product mix exiting the injector, m_i can be determined from a simple mass balance:

$$m_i = m_p + m_s \quad 5.4$$

where m_p is the flowrate of incoming milk and m_s is the flowrate of steam. Therefore, at a flowrate of 100 L/hr (with the previously described 70 °C preheat and 121 °C final heat conditions), the flowrate exiting the injector, $m_{i,}$ is 109.5 L/hr.

The thermodynamic conditions in the supersonic steam injector unit were analysed to understand the thermodynamic conditions in the deLaval nozzle (Fig. 5.3). While the mass flowrate is contant at successive sections of the nozzle, the volumetric flowrate changes as it goes through, mainly for two reasons (i) the cross-sectional area is changing, and (ii) steam is being condensed as it moves through the nozzle. The percentage of the total steam load that is condensed before the throat is not knowm and hence in the simulation this percentage is treated as an arbitrary constant which can be set between 0 and 100%. The velocity profile for steam at successive sections can be described by the steady flow enthalpy equation, based on the law of conservation of energy as applied to compressible fluids:

$$h_1 + v_1^2/2 = h_2 + v_2^2/2 \qquad 5.5$$

where h is the specific enthalpy of steam and v is the velocity of fluid flow. For cases in which v_1 is very small in comparison to v_2, v_1 may be neglected (Gupta, 2013), resulting in the equation:

$$v_2 = \sqrt{2(h_1 - h_2)} \qquad 5.6$$

For a system using saturated steam supply (6 bar pressure absolute) at nozzle inlet (1) and throat (2) pressure of 3 bar(a), we obtain using saturated steam tables, h_1 = 2756 kJ⁄kg, and near supersonic conditions, using wet steam tables, h_2= 2639 kJ⁄kg, giving

$$v_2 = 485\ m/s$$

The Mach number can be written as:

$$Ma = \frac{v}{c} \qquad 5.7$$

where c is the local speed of sound, 440 m/s. Thus, Ma at the throat is calculated as 1.1; however, as the flow would become choked at the throat, to a maximum of Ma = 1, the maximum throat velocity would equal c.

If the fluid flow reaches sonic velocity at the throat, the velocity increases in the diverging section of the de Laval nozzle, as the area increases, as described in equation 5.8 below (which is derived from the Bernoulli continuity equation in many textbooks), i.e. giving supersonic flow, since the combination of $Ma \geq 1$ and $dA > 0$ gives dv >0, hence increasing v:

$$\frac{dA}{A} = -\frac{dv}{v}(1 - Ma^2) \qquad 5.8$$

However, after supersonic flow has been achieved within the diffuser, boundary conditions will ensure that the velocity will eventually decrease at a point which depends on the ratio of inlet pressure to exit or back pressure of the injector and drop below Ma = 1 to subsonic flow. This irreversible adjustment of velocity results in a shock wave. It should be noted that achieving this condition depends on matching the nozzle dimensions to the flow of product and steam, using

$$Q = vA \qquad 5.9$$

where Q is the volumetric flowrate of steam at the throat and A is the available cross sectional area, with allowance for a slight restriction due to product flow determined by the densities of product and steam.

The mass flowrate of water removed from the steam-product mix, m_l, by flash cooling can be determined by calculating the weight percent vapourised, X:

$$X = \frac{100(h_u^L - h_d^L)}{h_d^v - h_d^L} \quad 5.10$$

where h^L is the liquid enthalpy upstream (u) and downstream (d) from the flash cooler, and h^v is the vapour enthalpy at the flash cooler.

The mass flowrate of vapourised liquid in the flash cooler, m_l, can be determined using:

$$m_l = X\left({}^{m_i}/_{100}\right) \quad 5.11$$

Best operating practices of direct heating systems recommend that the flash cooling system is operated so that the flash cooling temperature is equal to the preheat temperature used (Burton, 1968). For a product at 121 °C where the flash cooler is operated at 0.3 bar(a) resulting in a flash cooling temperature of 70 °C, the mass flowrate of liquid being removed from the flash cooler can be calculated as:

$$X = 11.40$$

$$m_l = 12.5\ kg/hr$$

Note that there is still a differential between the calculated values for m_s and m_l, which may result in a 2.98 % concentration of the product despite ideal operating conditions. As described by Burton and Lewis (2009), the total solids of the product should be

monitored for dilution or concentration and the temperature of the flash cooler adjusted if required, as dilution of product is commonly reported in pilot-scale operation of direct heating systems (Dickow *et al.*, 2012b; Murphy *et al.*, 2013; Dumpler *et al.*, 2017). For the SSIH system, increasing the flash cooling pressure to 0.6 bar(a), related to a temperature of 86 °C, could limit the change in product solids to a dilution of 0.16%. However, for flash cooler operation at 0.3 bar(a), a simple mass balance can determine the quantity of product leaving the flash cooler to be 97 kg/hr.

5.5. Results

5.5.1. *Total Solids*

The total solids content is significantly affected by the heat treatment technology applied, with SSIH treatment resulting in a statistically significant increase of 0.4 g/100g from the unheated and IND-treated milks (Table 5.1; $p < 0.001$).

5.5.2. *Particle size distribution*

The particle size of heat-treated skim milk was significantly affected by the type of heating technology applied ($p < 0.01$), with an interactive effect between heating technology and preheat temperature ($p < 0.001$; Fig. 5.4). This effect was evidenced in the different impact of preheat temperature on IND- and SSIH-treated milks. For IND-treated milks, the preheat temperature selected had no significant impact on the average particle size (on average 179 and 174 d.nm for 70 and 85 °C, respectively), while all SSIH milks had a lower average particle size when preheated to 70 °C compared to their 85 °C counterparts (on average 166 and 185 d.nm for 70 and 85 °C, respectively).

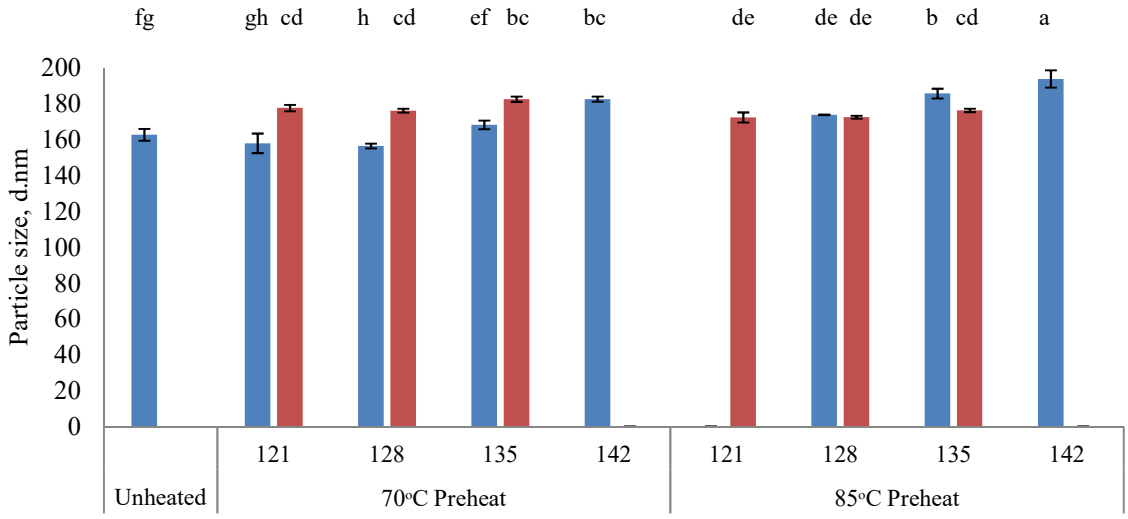

Fig. 5.4. Average particle size for (■) SSIH and (■) IND heat treated skim milk at a range of temperature-time combinations.

Final heat temperature also had a significant effect on the particle size of treated skim milks ($p < 0.001$). The particle size of all heat-treated milks increased significantly from the unheated control, with the exception of 70/121 °C and 70/135 °C SSIH milk. The particle size of SSIH-treated milks generally increased with increasing final heat temperature, while IND milks had a more consistent particle size across each of the final heat temperatures. Overall, the particle size of SSIH-treated milks was more heavily dependent on temperature than IND-treated milks.

Table 5.1. Physical characteristics of unheated, indirectly (IND) and directly (SSIH) heat treated skim milk[1]

Tech	Preheat	Final Heat	Total solids %	pH -	Viscosity mPa.s	L* -	a* -	b* -	ΔE -	Intability Index, %/hr
IND	Unheated		8.92 ± 0.13^{d}	6.79 ± 0.02^{a}	3.42 ± 0.05^{c}	84.35 ± 1.39^{d}	-7.66 ± 1.09^{abc}	6.12 ± 2.40^{a}	-	47.28 ± 1.96^{a}
	70°C	121°C	8.82 ± 0.03^{d}	6.79 ± 0.00^{ab}	3.42 ± 0.02^{bc}	86.86 ± 0.24^{cd}	-6.56 ± 0.05^{a}	6.54 ± 0.25^{a}	2.78 ± 0.34^{c}	51.64 ± 5.24^{a}
		128°C	8.96 ± 0.02^{cd}	6.78 ± 0.00^{ab}	3.38 ± 0.01^{c}	87.17 ± 0.12^{c}	-6.63 ± 0.15^{a}	7.53 ± 1.51^{a}	2.52 ± 0.84^{c}	46.81 ± 4.34^{a}
		135°C	8.87 ± 0.00^{d}	6.77 ± 0.00^{ab}	3.42 ± 0.02^{bc}	87.87 ± 0.32^{c}	-6.61 ± 0.08^{a}	6.77 ± 1.70^{a}	3.53 ± 0.70^{bc}	46.98 ± 2.68^{a}
		142°C	-	-	-	-	-	-	-	-
	85°C	121°C	8.95 ± 0.06^{cd}	6.79 ± 0.00^{ab}	3.37 ± 0.01^{c}	86.69 ± 0.41^{cd}	-6.61 ± 0.10^{a}	5.88 ± 1.32^{a}	3.32 ± 0.95^{bc}	50.33 ± 1.28^{a}
		128°C	8.83 ± 0.04^{d}	6.78 ± 0.00^{ab}	3.40 ± 0.01^{c}	87.42 ± 0.06^{c}	-6.73 ± 0.07^{ab}	8.02 ± 0.00^{a}	2.38 ± 0.05 ^{c}	48.15 ± 1.27^{a}
		135°C	9.00 ± 0.13^{bcd}	6.77 ± 0.00^{ab}	3.38 ± 0.01^{c}	88.25 ± 0.05^{c}	-6.57 ± 0.03^{a}	7.04 ± 1.30^{a}	3.61 ± 0.56^{bc}	47.35 ± 1.31^{a}
		142°C	-	-	-	-	-	-	-	-
SSIH	70°C	121°C	9.42 ± 0.10^{abc}	6.78 ± 0.00^{ab}	3.66 ± 0.06^{a}	87.07 ± 0.86^{cd}	-8.43 ± 0.00^{c}	6.60 ± 0.15^{a}	2.25 ± 0.67^{c}	52.97 ± 2.79^{a}
		128°C	9.22 ± 0.01^{abcd}	6.76 ± 0.00^{b}	3.73 ± 0.10^{a}	86.68 ± 1.44^{cd}	-8.44 ± 0.00^{c}	8.32 ± 2.98^{a}	5.86 ± 3.15^{abc}	47.73 ± 1.59^{a}
		135°C	9.29 ± 0.06^{abcd}	6.78 ± 0.00^{ab}	3.76 ± 0.10^{a}	88.76 ± 0.63^{bc}	-8.29 ± 0.22^{bc}	6.09 ± 0.24^{a}	3.69 ± 0.64^{bc}	49.47 ± 1.23^{a}
		142°C	9.47 ± 0.23^{ab}	6.77 ± 0.00^{ab}	3.72 ± 0.04^{a}	92.23 ± 0.27^{a}	-8.50 ± 0.02^{c}	8.16 ± 1.90^{a}	7.66 ± 0.89^{ab}	50.18 ± 1.43^{a}
	85°C	121°C	-	-	-	-	-	-	-	-
		128°C	8.85 ± 0.30^{d}	6.79 ± 0.00^{ab}	3.62 ± 0.06^{a}	86.67 ± 1.06^{cd}	-8.32 ± 0.13^{bc}	6.99 ± 1.76^{a}	4.94 ± 1.88^{abc}	52.50 ± 2.51^{a}
		135°C	9.51 ± 0.00^{a}	6.77 ± 0.00^{ab}	3.59 ± 0.06^{ab}	91.69 ± 0.09^{ab}	-8.47 ± 0.01^{c}	7.08 ± 0.65^{a}	6.76 ± 0.05^{abc}	50.64 ± 9.34^{a}
		142°C	9.62 ± 0.09^{a}	6.77 ± 0.01^{ab}	3.74 ± 0.02^{a}	92.97 ± 0.24^{a}	-8.52 ± 0.05^{c}	9.90 ± 0.42^{a}	8.98 ± 0.00^{a}	47.77 ± 1.84^{a}

[1] Mean values with a common superscript letter in the same column are not significantly different ($p < 0.05$)

5.5.3. *Viscosity*

The viscosity of IND-treated milk did not significantly differ from that of the unheated control (Table 5.1; ($p > 0.05$). However, SSIH-treated milks had a greater viscosity than their IND-treated counterparts, with statistically significant increases in viscosity (by around 6% versus the unheated control) ($p < 0.001$). Preheat and final heat temperature did not significantly affect the viscosity of IND- or SSIH-treated milks ($p > 0.05$).

5.5.4. *Whey protein denaturation*

The type of heating technology applied had a significant impact on the level of whey protein denaturation observed in the heat-treated skim milks for α-la ($p < 0.01$), β-lg A ($p < 0.001$), and β-lg B ($p < 0.001$) (Table 5.2). SSIH-treated milks retained a significantly greater level of native whey proteins for each whey protein fraction compared to IND-heating, most notably for heat-labile β-lg A and B. IND-treatment resulted in a near complete denaturation of β-lg A and B, reducing the native level to an average of 4.01% of the initial native content in the unheated control. Preheat temperature had a significant effect on the retention of native β-lg variants for SSIH-treated milks ($p < 0.05$). Milks preheated to 70 °C had a higher proportion of native β-lg A and B than those preheated to 85 °C.

Table 5.2. Level of whey protein denaturation in heat-treated skim milk represented as a percentage of the initial native protein content of the unheated skim milk

Tech	Preheat	Final Heat	α-Lactalbumin	β-Lactoglobulin	
				B	A
IND	70°C	121°C	84.3 ± 7.37^{abc}	4.10 ± 4.86^{d}	5.26 ± 6.09^{c}
		128°C	73.1 ± 7.54^{bc}	3.57 ± 0.82^{d}	8.53 ± 0.52^{c}
		135°C	48.9 ± 2.81^{e}	1.37 ± 1.37^{d}	0.83 ± 1.66^{c}
		142°C	-	-	-
	85°C	121°C	76.1 ± 2.47^{bc}	5.64 ± 1.03^{d}	9.05 ± 1.10^{c}
		128°C	65.6 ± 9.58^{cd}	2.91 ± 2.06^{d}	4.13 ± 4.79^{c}
		135°C	43.6 ± 3.82^{e}	2.67 ± 3.14^{d}	0.00 ± 0.00^{c}
		142°C	-	-	-
SSIH	70°C	121°C	90.4 ± 10.0^{ab}	80.9 ± 10.1^{a}	85.1 ± 7.99^{a}
		128°C	89.6 ± 11.2^{ab}	80.5 ± 7.44^{a}	85.7 ± 9.57^{a}
		135°C	93.1 ± 11.7^{ab}	80.5 ± 6.87^{a}	83.1 ± 8.90^{a}
		142°C	102 ± 10.7^{a}	71.8 ± 11.5^{ab}	76.2 ± 9.63^{a}
	85°C	121°C	-	-	-
		128°C	85.3 ± 7.03^{abc}	63.9 ± 2.48^{b}	71.9 ± 2.86^{ab}
		135°C	86.8 ± 9.57^{ab}	48.7 ± 5.41^{c}	58.6 ± 5.80^{b}
		142°C	- [1]	- [1]	- [1]

[1] The whey protein denaturation result for the 85/142 ºC treatment was removed due to a high standard deviation between the replicates.

5.5.5. *Colour analysis*

Lightness, L*, of heat-treated skim milk samples was not significantly affected by heating technology or preheat treatment ($p > 0.05$). However, the final heat temperature was shown to significantly affect the L* values for SSIH-heated milks, as L* increased with increasing final heat temperature.

Heating technology had a significant impact on the a* value, or red-greeness, of heat-treated skim milks ($p < 0.001$). While neither IND- or SSIH-treated samples were significantly different from the unheated control in terms of a* value, they were significantly different from each other ($p < 0.05$). All IND-treated samples were seen to increase in a* value as a result of heat treatment with an average a* value of -6.62, while SSIH-treated samples decreased with an average value of -8.42. There was no significant difference in b* value for any of the heat-treated formulations.

Euclidean colour difference, ΔE, was employed to quantify the overall colour difference in the skim milks due to heat treatment. ΔE was affected by the type of heating technology applied ($p < 0.05$), with SSIH-milks resulting in a higher ΔE than IND-milks overall. This difference in ΔE is most attributable to the changes in L* value. As for L* value, final heat temperature was also shown to impact ΔE for SSIH milks, as ΔE broadly increased with final heat temperature. It should be noted that while these colour measurements are consistent with other physico-chemical changes, no undesirable colour effects were visibly perceived.

5.5.6. *Accelerated physical instability*

Overall trend analysis of accelerated physical instability, using 3-way ANOVA, revealed that it was influenced by the type of heating technology applied ($p < 0.05$), with IND-milks having a lower instability index value compared to SSIH milks. However, Tukey *post hoc* analysis of individual samples did not show significant differences between individual heat-treated milks indicating that there is likely no meaningful difference in stability between the two heating technologies (Table 5.1; $p > 0.05$). The transmission profiles of 85/128 °C SSIH-treated milk showed increased transmission in the lower two thirds of the tube, 100 – 110 mm compared to IND-milk, indicating increased levels of sedimentation in the system (Fig. 5.5).

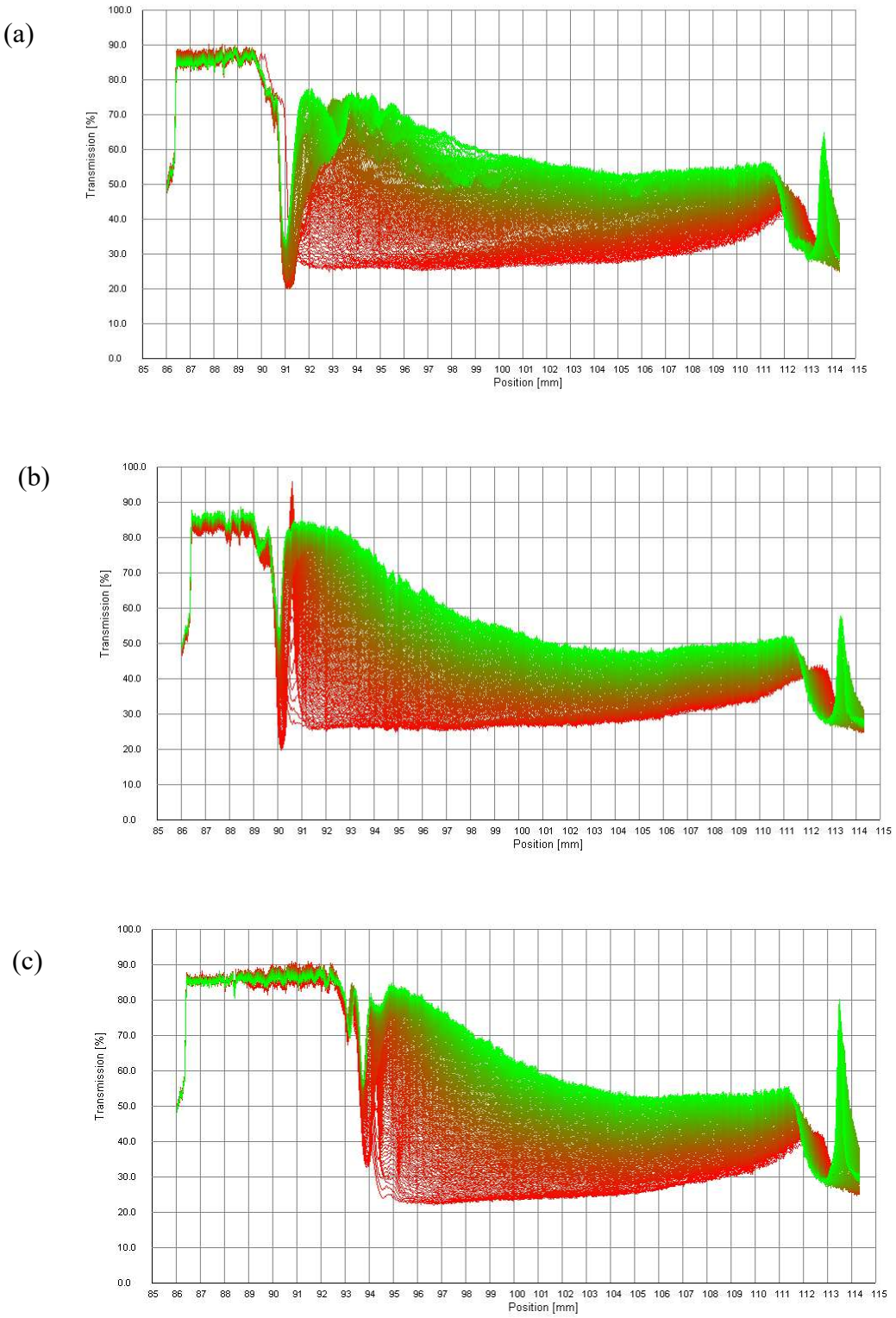

Fig. 5.5. Accelerated storage stability transmission profiles of (a) unheated control, (b) SSIH 85/128 °C and (c) IND 85/128 °C skim milk as a function of position (91 to 114mm) from 0 (red) to 7 hr 25 min (green).

5.6. Discussion

The changes in total solids content reported for SSIH-treated milks are commonly reported in direct heating pilot-scale operation (Dickow *et al.*, 2012a; Murphy *et al.*, 2013; Dumpler *et al.*, 2017; Kelleher *et al.*, 2018) and are associated with the operation of the flash cooler which removes excess condensed steam added during injection. The flash cooling pressure and associated temperature can be set to control the quantity of liquid removed by vaporisation and therefore the level of total solids in the product stream.

The impact of preheat temperature on particle size was found to be significant for SSIH-treated skim milks but not for IND-milks. Preheating milk in a UHT process has been shown to result in protein stabilisation and can improve thermal stability during final heat treatment, minimising changes in average particle size due to heat treatment (Prakash *et al.*, 2015; Drapala *et al.*, 2016, Dumpler and Kulozik, 2016; Joyce *et al.*, 2017). This study would indicate that a reduced degree of thermally-induced aggregation can be achieved through the application of the lower preheat temperature, 70°C, for SSIH-treated skim milk.

Viscosity, L* value and physical instability under accelerated conditions were all shown to be significantly higher for SSIH-treated milks compared to IND-milks. Each of these physical properties can be related to increased aggregate size, through Einstein's equation for viscosity (Anema and Li, 2003), Rayleigh's law for L* values (Chung *et al.*, 2014), and Stokes' law for physical instability (Chen and O'Mahony, 2016). Previous studies have shown that direct heat treatment of dairy products can result in the production of a small quantity of highly aggregated protein material, driving increases in characteristics such as particle size, viscosity, lightness, and even

sedimentation, despite reduced levels of protein denaturation as shown in this study (Dumpler *et al.*, 2016; Malmgren *et al.*, 2017; Kelleher *et al.*, 2018). These differences in impact between heat treatment technologies are likely related to differences in heating mechanisms and the knock-on effect of protein denaturation and aggregation on reaction kinetics (Anema and McKenna, 1996; Oldfield *et al.*, 1998; Joyce *et al.*, 2017). It has also been suggested that the reduced fouling widely reported for directly heated systems may be related to the presence of this highly aggregated material, as the aggregates that would generally adhere to the heat exchanger and foul during traditional indirect processing are instead present in the final product (Burton, 1968; Datta *et al.*, 2002).

5.7. Conclusion

SSIH technology had a significant impact on limiting the level of protein denaturation in skim milk compared to conventional IND technology, particularly for heat-labile β-lg A and B whey proteins. However, the application of the SSIH technology was also found to have a negative effect on some of the physical characteristics of the treated skim milk. Increases in particle size for SSIH milks had a knock-on effect with increased changes in colour, viscosity and sedimentation under accelerated conditions, compared to IND-treated milks. The SSIH technology would be useful to produce dairy products with significantly less thermal damage to the dairy proteins; however, more investigation is required to improve the overall stability of the final product.

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Chapter 6.

A comparison of pilot-scale supersonic direct steam injection to conventional steam infusion and tubular heating systems for the heat treatment of protein-enriched skim milk-based beverages

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Kelleher, C.M., Tobin, J.T., O'Mahony, J.A., Kelly, A.L., O'Callaghan, D.J, and McCarthy, N.A. (2018). A comparison of pilot-scale supersonic direct steam injection to conventional steam infusion and tubular heating systems for the heat treatment of protein-enriched skim milk-based beverages. Innovative Food Science and Emerging Technologies, Accepted

6.1. Abstract

Direct supersonic steam injection, direct steam infusion, and indirect tubular heating were each applied to protein-enriched skim milk-based beverages with 4, 6 and 8% (w/w) total protein, and the effect of final heat temperature on the physical properties of these beverages was investigated. Supersonic steam injection resulted in significantly lower levels of denaturation of β-lactoglobulin (34.5%), compared to both infusion (76.3%) and tubular (97.1%) heating technologies. Viscosity, particle size and accelerated physical stability of formulations did not differ significantly between the heating technologies, while noticeable colour differences due to heat treatment (mainly attributed to increasing b* value) were observed, particularly for tubular heating. Overall, the extent of protein denaturation in high-protein dairy products was significantly influenced by the particular heating technology applied. The application of supersonic steam injection technology, with rapid heating and high shear characteristics, may provide new product development opportunities for the processing of ready-to-drink ambient-delivery high-protein dairy beverages.

6.2. Introduction

Extended shelf life (ESL) and ultra-high temperature (UHT) treated milk have increased in popularity worldwide, providing long shelf-life and eliminating cold chain requirements, thereby reducing economic costs to producers, distributors and consumers (Bertolini *et al.*, 2016, Malmgren *et al.*, 2017). High-heat dairy treatments, like ESL heating (120-135 °C for 2-4 s) and UHT (135–145 °C for 2-4 s), can negatively impact the nutritional quality and sensory properties of the final product due to the severity of the heat treatment applied. In addition, the choice of thermal processing technology used to achieve ESL or UHT treatment can have a significant impact on physical properties and consumer acceptability of the final product (Deeth and Lewis, 2016; Roux *et al.*, 2016).

Thermal processing technologies may be classified as direct or indirect, which have different heat transfer mechanisms. With indirect heating, heat transferred through a thermally conducting barrier, while direct heating involves the addition of steam which heats the product by giving up its latent heat (Hsu, 1970; Burton, 1994; Schroyer, 1997; Lewis *et al.*, 2000). Direct heating is a more rapid and thermally efficient, form of heating than indirect heating, and imparts a lower thermal load on the product due to much faster heating and cooling (Kelleher et al., 2018b). However, there are challenges with direct systems such as the requirement for culinary-grade steam, lower heat regeneration capacity, and concerns with product dilution, resulting in indirect technologies being more commonly used industrially (Datta *et al.*, 2002; Britz and Robinson, 2008; Dickow *et al.*, 2012b; Karayannakidis *et al.*, 2014; Lee *et al.*, 2017).

High-velocity steam injection units have been developed, improving on the basic steam injection geometries first used (Ford *et al.*, 1969; Patrick and Swaisgood, 1976). This study used a patented supersonic steam injector (Maklad Fluid GmbH), which makes use of a De Laval nozzle to achieve better mixing and potentially attaining supersonic flow within the injection unit. De Laval nozzles were first developed in 1890 by Gustaf de Laval for use on a steam turbine and are commonly used today in rocket and jet engines. These nozzles are commonly referred to as converging-diverging nozzles, where an inlet section converges into a narrow throat and subsequently expands into a divergent outlet (Canosa et al., 2016). As explained in section 5.4, the converging inlet accelerates the fluid, in this case a mixture of steam and liquid product, until the flow becomes choked or sonic at the throat, where the cross-sectional area is smallest, and a Mach number (Ma) of 1 is achieved. Due to conservation of volumetric flow, temperature and pressure reduce with increasing area in the diverging outlet, thereby increasing kinetic energy and resulting in supersonic flow (Ma > 1). The application of a supersonic steam injector in dairy processing can theoretically produce better product mixing due to high shear from (i) the high throat velocities and (ii) the shockwave produced, with reduced residence time in the injection chamber, compared to traditional direct systems in dairy processing (Murphy *et al.*, 2011; Murphy *et al.*, 2013).

Increased consumer awareness has led to market demands for healthy, protein-enriched foods for general consumption, in addition to clinical uses such as for the treatment of malnutrition, sarcopenia in the elderly, high-performance sports nutrition, and body-building (Hayes *et al.*, 2008; Jelen, 2009; Shiby, 2013; Withers *et al.*, 2014; Chen and O'Mahony, 2016). Milk proteins have many health-promoting and

nutritionally beneficial properties for the consumer, such as supplying essential amino acids for tissue growth and repair, metabolic regulation for weight control, and anti-oxidant functions for immune-enhancing properties (Beucler, 2005; Smithers; 2008; Wijayanti *et al.*, 2014; Gupta and Prakash, 2015). However, protein-enriched beverages can pose thermal processing challenges, particularly in relation to the denaturation, aggregation and fouling of heat-labile dairy proteins, with the selection of thermal processing technology having a significant impact on the occurrence of these phenomena (Joyce *et al.*, 2017; Kelleher *et al.*, 2018). The nutritional value of proteins can be impaired by severe heat treatment, resulting in decreased protein digestibility and the availability of substrate to enzymatic digestion (Resmini *et al.*, 2003). 'Cooked' off-flavours commonly associated with ESL and UHT milks are connected to the level of whey protein denaturation, particularly β-lactoglobulin, as free sulfhydryl groups are exposed leading to the development of sulphur compounds in the milk (Al-Attabi *et al.*, 2009; Zabbia *et al.*, 2012; Lee *et al.*, 2017). Incorporation of ingredients can also pose challenges in high protein beverage systems, with commonly used powder ingredients such as milk protein concentrates (MPC) exhibiting poor solubility. The application of high temperatures, shear and increased hydration time can improve MPC solubility and incorporation into beverage formulations (Pathania *et al.*, 2018). Novel thermal processing technologies may prove to be important tools for the food industry in the development of protein-enriched beverages with differentiated physical properties which can satisfy changing market demands.

The aim of this study was to investigate the impact of direct supersonic steam injection heating on the physical characteristics of ready-to-drink protein-enriched dairy-based beverages with ambient distribution, compared to standard direct infusion and indirect

tubular heating technologies. The three heating technologies were applied to beverages having three different protein levels, operated at three final heat treatment temperatures, and compared in terms of final product quality and stability. As little has been published in relation to the use of supersonic injectors in dairy processing, the focus of this study was to determine the implications of high shear and rapid heat transfer during processing using the supersonic injector and analysing for protein denaturation, and beverage viscosity and physical stability.

6.3. Materials and methods

6.3.1. *Materials and formulation*

Medium-heat skim milk powder, SMP (33.93% protein, 0.78% fat, 6.04% moisture, 48.88% lactose and 8.13% ash), and milk protein concentrate, MPC80 (83.03% protein, 0.96% fat, 4.03% moisture, 3.99% lactose and 6.96% ash) were supplied by Glanbia Ingredients Ireland Ltd. (Kilkenny, Ireland).

Model protein-enriched beverages were formulated at 4%, 6% and 8% w/w protein concentrations using a skim milk base, reconstituted to 10% total solids (w/w) in reverse osmosis water at 45 °C using a YTRON ZC powder induction unit (YTRON Process Technology GmbH, Bad Endorf, Germany). MPC80 was added to each formulation to yield desired protein concentration (0.73, 3.14, 5.55 % MPC80 (w/w) for 4, 6 and 8 %, respectively) and inducted with a high shear mixer (Silverson EX, Silverson Machines Ltd, UK). This resulted in formulations with an 80:20 casein to whey protein ratio and a total solids content which increased with increasing protein content. The formulations were held overnight in stirred tanks at 4°C to allow for powder hydration. The pH was measured before and after overnight storage and was adjusted to pH 6.7 using 0.1 M HCl or KOH, if required.

6.3.2. *Heat Treatment*

Three types of heat treatment technology were applied to the formulations: direct steam infusion, direct steam injection, and indirect tubular heating (Fig. 6.1). All heat treatment conditions consisted of a preheat treatment (70°C for 30 s) and final heat treatment (121°C, 135°C or 142°C, for 3s) with a flowrate of 100 L/hr. Indirect tubular heating was applied using a MicroThermics tubular UHT 25HV pilot plant (MicroThermics, NC, USA), consisting of four tubular heat exchangers; preheat, final heat and two cooling exchangers. The residence time within a tubular heat exchanger for the MicroThermics system is 30s at 100 L/hr. The direct steam injection was achieved by integrating a purpose-built process line with a Maklad supersonic injector Model 700-143-60 (Maklad Innovative Fluid- and Systemtechnik GmbH, Austria) into the MicroThermics plant for final heat operation. Vacuum flash cooling to 70 °C was applied after final heat treatment as part of the injection process line, while the MicroThermics tubular heaters were used for preheating and final cooling operations. The residence time for the tubular heating, steam injection and flash cooling operations at 100 L/hr were taken as 30s, 1s and 1s, respectively. The injector had a de Laval converging-diverging nozzle with a flow rate range of 50 – 150 L/hr and had a Teflon coating in the steam-product mixing zone to reduce burn-on. The process line consisted of the Maklad injector, flash cooler, condenser, product pumps, culinary steam and product filters and an independent cleaning-in place (CIP) system. Infusion heating employed an UHT pilot-scale plate exchanger Model 422463 (APV, Denmark), and as in the injection system, initial cooling to 70 °C was achieved using vacuum flash cooling. Preheat and final cooling operations were carried out using plate heat exchangers. The residence time for the plate preheat, steam infusion and flash cooling operations at 100L/hr were taken as 60, 3 and 1s, respectively. All heat

treatment trials were carried out in triplicate. A calculation of the F_0 value, a bacterial lethality index, was completed for the three heat treatment technologies to ensure the differences in thermal load, due to heating and cooling times, did not significantly impact the predicted decimal reduction of bacterial organisms and comparability of the systems. The F_0 was determined using the following equation:

$$F = \int_0^\infty 10^{(\theta-\theta_{ref})/z} . \mathrm{dt} \qquad 6.1$$

Where the reference temperature, θ_{ref}, is 121.1 ºC and the z-value is 10 ºC as determined for *Clostridium botulinum* spores (Lewis and Heppell, 2000). The F_0 values determined for the three heat treatment technologies were comparable across each of the final heat temperatures applied with ranges of 0.49 – 0.51 for 121ºC final heat, 12.25 – 12.65 for 135ºC final heat and 61.37 – 63.41 for 142ºC final heat. However, it should be noted that the difference in F_0 increases with increasing final heat tempeature, particularly for 142ºC

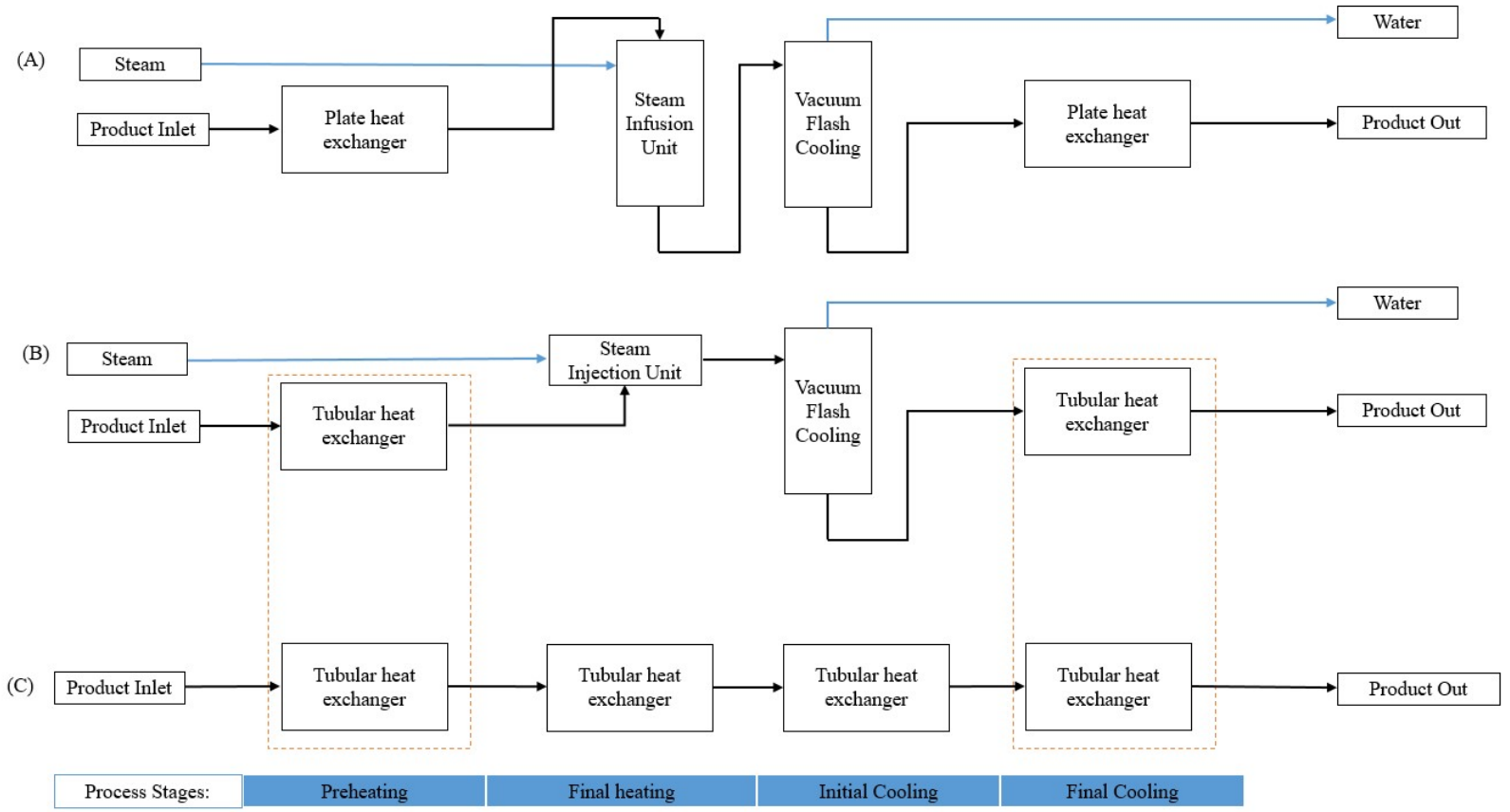

Fig. 6.1. Process flow diagram of (A) direct steam infusion, (B) direct steam injection and (C) indirect tubular heat exchange pilot plants across preheating, final heating, initial and final cooling operations. Common preheat and final cooling operations are used for the direct injection and indirect tubular plants (----).

6.3.3. Protein analysis

Total protein content was determined using the Kjeldhal method with a nitrogen-to-protein conversion factor of 6.38 (IDF, 2001). Native protein levels were determined using reverse-phase high-performance liquid chromatography (RP-HPLC) equipped with a Waters 2695 separation module, Waters 2487 dual wavelength absorbance detector at 214 nm and Empower® software (Milford, MA, USA). The HPLC was equipped with a PolymerX 5µm RP-1, 150x4.6mm column (Phenomenex, Cheshire, UK). α-Lactalbumin (α-la), β-lactoglobulin A and B (β-lg A and B) standards (Sigma Aldrich, Ireland) were used to calibrate the method. Sample preparation required pH adjustment to 4.6 with 0.1 M acetate buffer to 2.5 $g.L^{-1}$ protein, centrifugation at 20,000g for 20 min at 4 °C, and filtration of the supernatant using 0.2 µm PES filters (Agilent Technologies, California, United States) (Kehoe *et al.*, 2011; Kelleher *et al.*, 2018). Total solids content was measured using a Smart System 5, Smart Trac (CEM Corporation, Matthews, NC, USA).

6.3.4. *Viscosity*

Viscosity was determined using a shear rate sweep at 25°C, using an AR-G2 controlled stress rheometer (TA Instruments, Crawley, UK) with a concentric cylinder geometry (Murphy *et al.*, 2013). The shear rate sweep consisted of a ramp from 0–500 s^{-1} over 5 min, a hold at 500s^{-1} for 1 min, and a ramp from 500–0s^{-1} over 5 min. The apparent viscosity values presented are the average viscosity on holding at 500 s^{-1} for 1 min.

6.3.5. *Particle size*

A Malvern Zetasizer Nano ZS combined dynamic, static and electrophoretic light scattering analyser (Malvern Instruments Ltd., UK) was used to determine particle size at 25°C. Samples were dispersed using ultra-pure water in polystyrene disposable

cuvettes with refractive index for protein and the water dispersant of 1.45 and 1.33, respectively.

6.3.6. *Colour analysis*

Colour of samples in disposable cuvettes was measured using a Minolta Chroma meter CR-400 colorimeter (Minolta Ltd., Milton Keynes, UK) and expressd in with L*, a* and b* values. A white plate (Y, x and y of 88.3, 0.316 and 0.3226, respectively) was used to calibrate the colorimeter. Colour difference from unheated formulations, ΔE, was determined using the CIE76 Euclidean distance formula, given as described by (Morales and Jiménez-Pérez, 2001):

$$\Delta E = \sqrt{(L_2^* - L_1^*)^2 + (a_2^* - a_1^*)^2 + (b_2^* - b_1^*)^2} \quad 6.1$$

6.3.7. *Accelerated physical stability*

Accelerated physical stability of formulations was investigated using a LUMiSizer analytical centrifuge (Lum GMBH, Berlin, Germany), equipped with SepView 4.1 software. Samples (0.4 mL) were filled into PC100-131XX polycarbonate cells to a 20 mm depth and centrifuged at 2300g for 3 h at 25 °C (Chen and O'Mahony, 2016). The software integrates with respect to particle position on each transmission profile to characterise instability over time as a second order polynomial. To calculate the change in transmission over time, integration limits were set along the length of the filled tube, at 110 to 130 mm. The average slope of this polynomial, calculated from the polynomial coefficients, was used as an instability index to compare the stability of different samples under accelerated conditions.

6.3.8. *Statistical analysis*

Heat treatment trials were carried out in triplicate. Three-way ANOVA was carried out to determine levels of statistical significance of protein content, heating

technology and heat treatment temperatures, and interactions between these factors; one-way ANOVA with Tukey *post hoc* analysis was used to detect significant differences in treatment means at $p < 0.05$, using Minitab® 17 (Minitab Ltd., Coventry, UK). Results and discussion

6.3.9. *Protein analysis*

Total solids and total protein were analysed before and after heat treatment and steam injection and tubular heat treatment did not significantly differ in their effects on the level of total solids and total protein for 4, 6 and 8% (w/w) protein formulations (Table 6.1). However, steam infusion technology resulted in a significant reduction in total solids and protein levels at each protein concentration and treatment temperature applied ($p < 0.001$; Table 6.2.). This reduction in total solids content during thermal processing may be due to the deposition of some denatured material due to fouling in the system. This fouling could take the form of type A fouling, involving the depositions consisting of 60-70 % protein at temperatures between 75 and 110 °C, and type B fouling, involving depositions of 70-80 % mineals at temperatures greater than 110 °C (Bansal and Chen, 2006). It should be noted however, that for direct heating systems, reductions in solids content are also commonly reported and are caused by product dilution by the condensed steam heating medium, due to insufficient removal of water by flash cooling (Dickow *et al.*, 2012a; Dickow *et al.*, 2012b; Murphy *et al.*, 2013; Dumpler *et al.*, 2017). The application of injection and tubular heating resulted in no significant change in total protein content being observed and while infusion treatment affected the level of total protein in the final formulation (possibly due to fouling), injection heating did not.

The level of native protein was significantly affected by the type of heating technology, protein concentration and temperature applied ($p < 0.001$, $p < 0.001$ and $p < 0.05$, respectively; Table 6.2.). The concentrations of native α-la were most greatly reduced by tubular heating, resulting in a final native α-la content that was 11.7-55.9% of the initial content in unheated formulations. Both direct injection and infusion heating resulted in significantly less denaturation of α-la, compared to tubular heating. For infusion technology, treatment at 121 °C did not affect the level of native α-la at any concentration ($p > 0.05$), while higher temperatures (135 and 142 °C) resulted in a reduction in levels of native α-la (on average 65.8% native α-la from the initial content; $p < 0.05$). Injection heating resulted in the lowest level of α-la denaturation of all technologies investigated, with no significant change in native α-la level (on average 82% native α-la from the initial content; $p > 0.05$) despite the application of high temperatures, with the exception of the treatment at 135 °C for the 4% w/w protein formulation (with 71% native α-la from the initial content; $p < 0.05$).

Despite extensive denaturation of β-lactoglobulin (β-lg) for all heat treatments, significant differences between heating technologies were evident (Fig. 6.2). Injection heating resulted in heat-treated formulations with significantly greater levels of native β-Lg A and B, compared to tubular and infusion heating ($p < 0.001$; Table 6.2). The average levels of native β-lg A and B were greater after injection (64.7 and 66.4%, respectively) compared to tubular heating (3.16 and 2.72%, respectively), indicating substantial denaturation with the use of indirect tubular heating. For 4% (w/w) protein formulations, the differences between infusion and tubular heating were statistically significant ($p < 0.05$); however, at higher protein concentrations, these differences were not significant.

Table 6.1. Physico-chemical characteristics of protein-enriched skim milk beverage formulations at 4, 6, and 8% protein before and after heat treatment with direct infusion, direct injection or indirect tubular heat treatment at a preheat temperature of 70°C for 30 s and final heat temperatures of 121, 135 and 142°C for 3s.[1]

	Treatment		pH	Total Solids	Total Protein	Viscosity	Particle Size diameter[2]
	Technology	Temp	-	% (w/w)	% (w/w)	m.Pas	(nm)
4% Protein	Unheated		6.74 ± 0.05^{a}	10.3 ± 0.09^{a}	4.02 ± 0.16^{ab}	3.86 ± 0.12^{a}	222 ± 8^{a}
	Infusion	121	6.75 ± 0.05^{a}	9.15 ± 0.09^{b}	3.82 ± 0.05^{bc}	3.83 ± 0.06^{a}	220 ± 11^{a}
		135	6.75 ± 0.07^{a}	9.09 ± 0.00^{b}	3.74 ± 0.03^{c}	3.86 ± 0.14^{a}	212 ± 1^{a}
		142	6.76 ± 0.06^{a}	8.94 ± 0.14^{b}	3.73 ± 0.07^{c}	3.86 ± 0.04^{a}	211 ± 2^{a}
	Injection	121	6.68 ± 0.02^{a}	10.1 ± 0.01^{a}	3.95 ± 0.06^{abc}	3.64 ± 0.09^{a}	238 ± 4^{a}
		135	6.68 ± 0.02^{a}	10.0 ± 0.0^{a}	3.92 ± 0.03^{abc}	3.72 ± 0.05^{a}	222 ± 9^{a}
		142	6.68 ± 0.01^{a}	10.1 ± 0.02^{a}	3.93 ± 0.01^{abc}	3.67 ± 0.03^{a}	280 ± 9^{a}
	Tubular	121	6.76 ± 0.06^{a}	10.1 ± 0.08^{a}	3.99 ± 0.19^{abc}	3.82 ± 0.10^{a}	205 ± 1^{a}
		135	6.74 ± 0.06^{a}	10.1 ± 0.08^{a}	4.13 ± 0.05^{a}	3.73 ± 0.17^{a}	214 ± 8^{a}
		142	6.74 ± 0.05^{a}	10.1 ± 0.03^{a}	4.09 ± 0.05^{ab}	3.82 ± 0.07^{a}	230 ± 10^{a}
6% Protein	Unheated		6.73 ± 0.06^{a}	12.3 ± 0.13^{a}	5.81 ± 0.63^{abc}	4.33 ± 0.16^{a}	291 ± 75^{a}
	Infusion	121	6.73 ± 0.09^{a}	11.5 ± 0.12^{b}	5.51 ± 0.08^{abc}	4.21 ± 0.21^{a}	238 ± 6^{a}
		135	6.73 ± 0.08^{a}	11.1 ± 0.05^{bc}	5.41 ± 0.16^{c}	4.10 ± 0.02^{a}	230 ± 8^{a}
		142	6.73 ± 0.06^{a}	11.0 ± 0.13^{c}	5.41 ± 0.09^{bc}	4.36 ± 0.12^{a}	231 ± 10^{a}
	Injection	121	6.64 ± 0.02^{a}	12.1 ± 0.03^{a}	5.95 ± 0.01^{abc}	4.25 ± 0.06^{a}	259 ± 4^{a}
		135	6.64 ± 0.02^{a}	12.1 ± 0.03^{a}	5.95 ± 0.01^{abc}	4.19 ± 0.04^{a}	250 ± 1^{a}
		142	6.64 ± 0.01^{a}	12.1 ± 0.06^{a}	5.95 ± 0.04^{abc}	4.27 ± 0.06^{a}	282 ± 8^{a}
	Tubular	121	6.72 ± 0.08^{a}	12.2 ± 0.09^{a}	5.93 ± 0.29^{a}	4.11 ± 0.14^{a}	227 ± 26^{a}
		135	6.71 ± 0.08^{a}	12.1 ± 0.25^{a}	5.88 ± 0.16^{abc}	4.18 ± 0.06^{a}	269 ± 48^{a}
		142	6.67 ± 0.07^{a}	12.1 ± 0.12^{a}	5.91 ± 0.20^{abc}	4.32 ± 0.05^{a}	272 ± 12^{a}
8% Protein	Unheated		6.69 ± 0.06^{a}	14.5 ± 0.12^{a}	7.75 ± 0.38^{a}	6.66 ± 1.33^{a}	366 ± 14^{a}
	Infusion	121	6.70 ± 0.08^{a}	12.8 ± 0.28^{b}	7.29 ± 0.30^{a}	4.69 ± 0.52^{b}	286 ± 6^{bc}
		135	6.72 ± 0.06^{a}	12.8 ± 0.29^{b}	7.25 ± 0.23^{a}	4.57 ± 0.45^{b}	284 ± 26^{bc}
		142	6.72 ± 0.06^{a}	12.7 ± 0.27^{b}	7.34 ± 0.20^{a}	4.66 ± 0.06^{b}	286 ± 10^{bc}
	Injection	121	6.62 ± 0.01^{a}	14.5 ± 0.06^{a}	7.88 ± 0.08^{a}	4.59 ± 0.13^{b}	282 ± 17^{bc}
		135	6.62 ± 0.00^{a}	14.4 ± 0.07^{a}	7.85 ± 0.03^{a}	4.94 ± 0.48^{b}	250 ± 6^{c}
		142	6.63 ± 0.00^{a}	14.2 ± 0.06^{a}	7.82 ± 0.07^{a}	4.66 ± 0.13^{b}	316 ± 20^{ab}
	Tubular	121	6.72 ± 0.06^{a}	14.4 ± 0.20^{a}	7.68 ± 0.47^{a}	5.15 ± 0.19^{b}	274 ± 20^{bc}
		135	6.69 ± 0.06^{a}	14.4 ± 0.19^{a}	7.73 ± 0.44^{a}	4.88 ± 0.43^{b}	289 ± 13^{bc}
		142	6.65 ± 0.05^{a}	14.2 ± 0.08^{a}	7.70 ± 0.51^{a}	4.79 ± 0.26^{b}	284 ± 13^{bc}

[1] For each formulation (protein concentration), mean values with a common superscript letter in the same column are not significantly different ($p > 0.05$).
[2] Average particle size is presented in terms of intensity mean.

Table 6.2. Statistical significance of the effects of target protein level, heating technology, severity of heat treatment and interactions of these factors on the physicochemical characteristics of heat treated solutions, assessed by three-way ANOVA[1].

Characteristic		Protein	Technology	Treatment	Protein* Technology	Protein* Treatment	Technology* Treatment
pH		**	***	NS	NS	NS	NS
Total Solids		***	***	**	***	NS	NS
Total Protein		***	***	NS	NS	NS	NS
Soluble Protein		***	***	*	***	NS	NS
Viscosity		***	NS	NS	NS	NS	NS
Particle Size		***	NS	***	*	NS	**
Colour	L*	***	***	***	NS	NS	NS
	a*	***	**	*	***	NS	**
	b*	***	***	***	*	NS	***
	ΔE	***	***	***	***	NS	***
Native Protein	α-La	***	***	***	NS	NS	*
	β-Lg B	***	***	NS	***	NS	NS
	β-Lg A	***	***	*	***	NS	NS

[1] *** indicates $p < 0.001$, ** indicates $p < 0.01$, * indicates $p < 0.05$ and NS indicates no significant difference.

While both infusion and injection systems resulted in higher levels of native protein than indirect tubular heating, supersonic injection technology resulted in the lowest whey protein denaturation levels at high processing temperatures. This may be due to the accelerated product flow within the injector chamber for supersonic injection, allowing the required heat to be imparted with reduced residence time, reduced thermal load and more uniform temperature (Murphy *et al.*, 2011; Murphy *et al.*, 2013). The application of high levels of shear, due to shockwaves produced in the supersonic injection system, may also contribute to the lower levels of protein denaturation observed. It has been shown that high shear does not contribute to protein denaturation and can instead reduce whey protein aggregate formation leading to greater retention of native protein post heat treatment (Walstra, 2002; Dissanayake and Vasiljevic, 2009; Çakır-Fuller, 2015; Wolz *et al.*, 2016). High shear within the system alters the type of flow and therefore the type of protein aggregation due to differences in particle collision. In addition to diffusion-controlled, perikenetic

collision resulting from the random motion of particles, hydrodynamic shear results in orthokinetic collison in which particles follow streamlines and colloide within shear flow impacting protein aggregation. At these high shear rates, the contact time between protein particles is short reducing the time for aggregate bond creation and therefore the number of aggregates created. Higher levels of shear stress can also fracture the weak spots in large agglomerates (Huppertz et al., 2019). Reduced degree of β-lg denaturation have been shown to reduce the levels of 'cooked' off flavours and sulphur volatiles in milk (Lee et al., 2017; Kelleher et al., 2018b). The substantially lower levels of denatured whey protein in injection-heated formulations is a significant differentiating attribute (e.g. with respect to sensory, colloidal stability and protein quality) for the final product, compared to infusion- and tubular-heated formulations.

6.3.10. *Viscosity*

While beverage viscosity increased with increasing protein and total solids content ($p < 0.001$), heat-treated formulations was not significantly affected by heating technology or temperature *per se* ($p > 0.05$; Table 6.1). For 4 and 6% (w/w) protein formulations, viscosity was not significantly affected by heat treatment using infusion, injection or tubular heating. Heat treatment significantly reduced the viscosity of 8% (w/w) protein formulations in all cases, by an average of 28.4% ($p < 0.05$) relative to the unheated formulation, with no significant effect of increasing heat treatment temperature or technology. This reduction in viscosity may be due to increased solubilisation of the added MPC powder at the high heat treatment temperatures for the more concentrated 8% (w/w) protein formulation as reported by Pathania *et al.* (2018).

6.3.11. *Particle size*

The average particle size for 4 and 6% (w/w) protein formulations was not significantly affected by heat treatment ($p > 0.05$; Table 6.1). For 8% (w/w) formulations, the unheated and injection 142 °C heated formulations resulted in the greatest particle size. This increase in the unheated formulation is likely due to the dissolution of MPC, an ingredient which is notoriously difficult to fully solubilise under standard processing conditions (McCarthy et al., 2014). With the application of heat treatment and, in the case of steam injection, shear effects, solubilisation of the MPC is improved and the average particle size is reduced for 8% (w/w) formulations.

While, overall, the average particle size did not differentiate significantly between heating technologies for most protein formulations, differences in particle size distribution were observed (Fig. 6.3). Injection heating resulted in a broader size distribution than infusion and tubular heating, for each treatment temperature and protein concentration. The high levels of shear produced by the supersonic injector may be the cause of the broadening distribution, as the degree of protein aggregation is reduced, and a higher quantity of smaller soluble aggregates are present in the system (Wolz *et al.*, 2016).

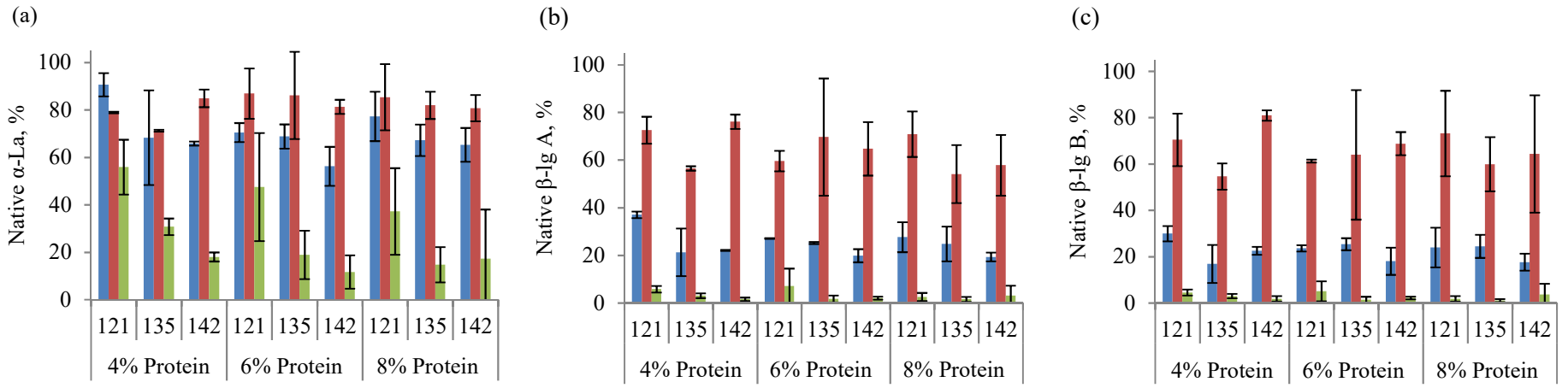

Fig. 6.2. Levels of native (a) α-la, (b) β-lg A and (c) β-lg B protein in 4, 6, and 8% protein (w/w) formulations heat-treated using direct steam infusion (■), direct steam injection (■) and indirect tubular heating (■) at final heat temperatures of 121, 135 and 142 °C, expressed as a percentage of the respective native protein content of the unheated formulation. The error bars represent the standard error determined from three trial replicates.

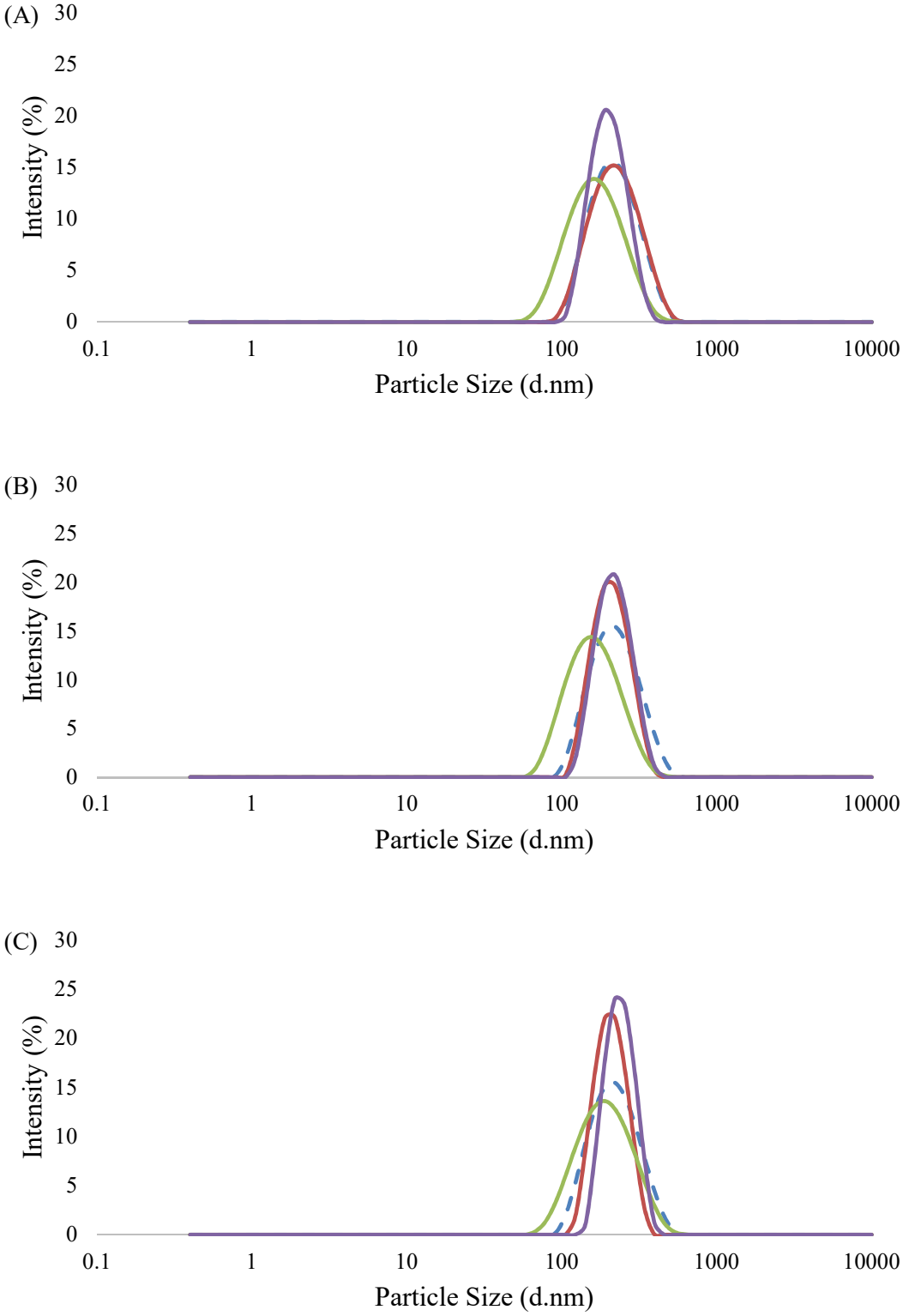

Fig. 6.3. Particle size distribution, on an intensity basis, of unheated (– – –) beverages, and infusion (——), injection (——), and tubular (——) heated 4% (w/w) protein formulation at final heat temperatures of (A) 121, (B) 135 and (C) 142 °C.

6.3.12. *Accelerated storage stability*

The level of protein had a significant impact on stability of formulations, with increasing protein concentration resulting in improved accelerated storage stability (Fig. 6.4). This is likely due to the increase in viscosity with increasing protein concentration (Table 6.1), which reduces particle settling velocity as per Stoke's Law (Lim and Roos, 2015). As with viscosity and particle size, there was no significant difference in accelerated storage stability of unheated, infusion-heated, tubular-heated or injection-heated formulations at any protein concentration ($p > 0.05$), while there were consistent trends for those parameters across protein levels

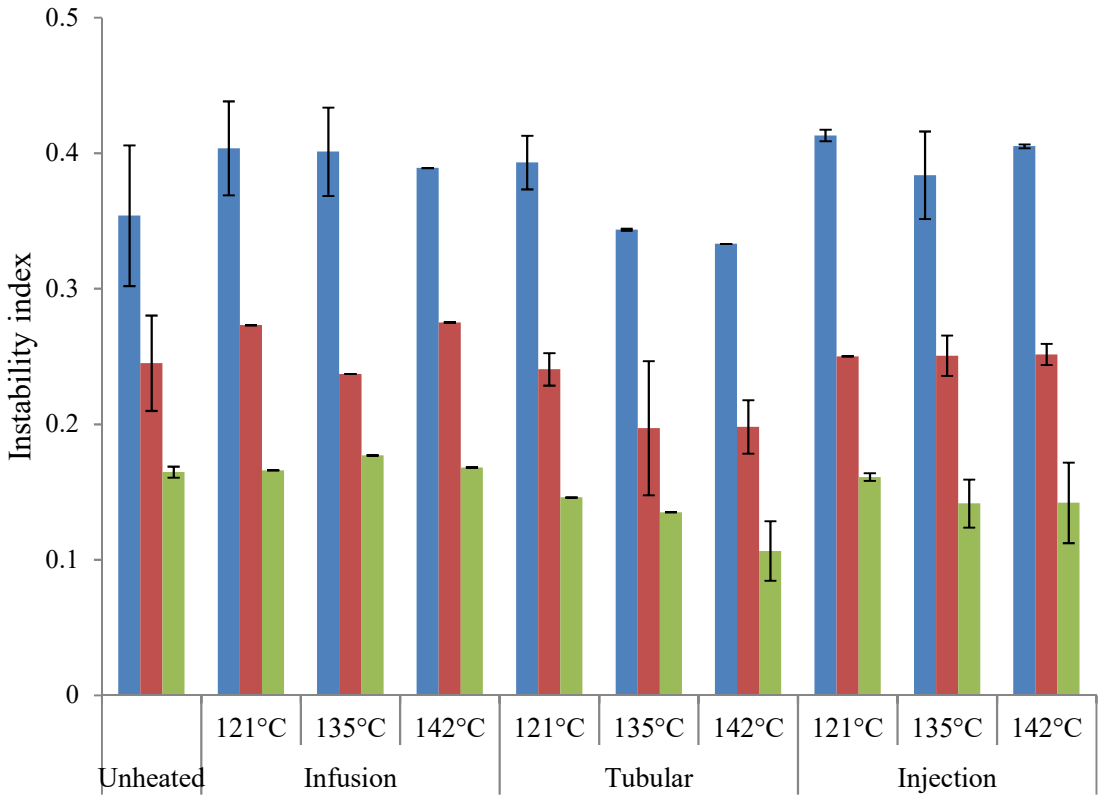

Fig. 6.4. Instability index of formulations, following accelerated storage stability using an analytical centrifuge at 2300 x g, for 3h at 25°C, with 4 (■), 6 (■), and 8% (■) protein (w/w) before and after heat treatment with direct infusion, direct injection or indirect tubular heat treatment at final heat temperatures of 121, 135 and 142 °C for 3 s.

6.3.13. *Colour analysis*

The protein content of formulations was found to have a significant effect on the lightness, L*, which increased in unheated formulations as the total solids and protein content increased ($p < 0.001$; Table 6.2 and 6.3). The L* value is largely attributed to particle size and total solids; therefore, as the level of casein micelles increases with protein concentration the L* value increases for the unheated formulations (Chung *et al.*, 2014). Heating technology had a significant effect on L* values of formulations ($p < 0.001$), with tubular heating resulting in a higher L* value for all 6% and 8% protein formulations compared to other heating technologies. Similar L* values were obtained for the 4, 6 and 8% protein formulations using infusion and injection direct heating systems (Table 6.3).

The a* value (red-greenness) of beverages generally increased upon heat treatment, an effect that become more significant with increasing protein concentration ($p < 0.001$; Table 6.2). At 4% protein, significant increases in a* value were identified for infusion treatments at 121 °C and 135 °C and tubular treatment at 142 °C only (Table 6.3). Heating technology and temperature were shown to affect a* value ($p < 0.01$ and 0.05, respectively), with tubular heating causing the greatest increase. Changes in a* value increased with increasing heating temperature. There was no significant difference between a* values following injection and infusion for the protein concentrations investigated.

The b* value increased with increasing protein concentration (Table 6.3). Heat treatment had a significant impact on the b* value, with infusion resulting in the lowest b* value and tubular treatment resulting in the highest b* value overall ($p < 0.001$;

Table 6.2). Tubular and injection heating significantly increased the b* value with increasing

Table 6.3. Colour analysis of protein–enriched skim milks at 4, 6 and 8 % protein (w/w) before and after heat treatment with direct infusion, direct injection or indirect tubular heat treatment at a preheat temperature of 70°C for 30 s and final heat temperatures of 121, 135 and 142°C for 3s.

	Tech.	Temp. (°C)	L*	a*	b*	ΔE
4% Protein	Unheated		75.44 ± 1.12^{a}	-5.32 ± 0.16^{c}	-0.23 ± 0.20^{d}	
	Infusion	121	75.54 ± 1.51^{a}	-4.75 ± 0.05^{ab}	-0.17 ± 0.17^{d}	0.91 ± 0.18^{c}
		135	76.15 ± 1.39^{a}	-4.86 ± 0.03^{ab}	-0.18 ± 0.39^{d}	0.92 ± 0.28^{c}
		142	76.95 ± 0.89^{a}	-5.06 ± 0.18^{abc}	0.18 ± 0.10^{d}	1.64 ± 0.29^{c}
	Injection	121	75.81 ± 0.13^{a}	-5.08 ± 0.02^{abc}	2.62 ± 0.04^{ab}	2.89 ± 0.09^{b}
		135	76.09 ± 0.39^{a}	-5.05 ± 0.07^{abc}	2.71 ± 0.02^{ab}	3.11 ± 0.24^{b}
		142	76.52 ± 0.05^{a}	-5.05 ± 0.03^{abc}	2.87 ± 0.02^{ab}	3.35 ± 0.09^{b}
	Tubular	121	77.07 ± 1.04^{a}	-5.18 ± 0.07^{bc}	0.43 ± 0.27^{cd}	1.77 ± 0.07^{c}
		135	77.75 ± 1.03^{a}	-5.17 ± 0.10^{bc}	1.68 ± 0.46^{bc}	3.01 ± 0.45^{b}
		142	78.80 ± 1.42^{a}	-4.70 ± 0.25^{a}	3.32 ± 0.73^{a}	4.95 ± 0.62^{a}
6% Protein	Unheated		78.08 ± 1.16^{b}	-5.05 ± 0.56^{b}	1.56 ± 0.43^{c}	
	Infusion	121	78.64 ± 0.58^{b}	-4.72 ± 0.19^{ab}	3.00 ± 1.11^{bc}	2.26 ± 1.56^{b}
		135	78.70 ± 0.48^{b}	-4.63 ± 0.21^{ab}	2.75 ± 0.97^{bc}	2.15 ± 1.40^{b}
		142	80.20 ± 1.37^{ab}	-4.66 ± 0.08^{ab}	2.65 ± 0.44^{bc}	2.34 ± 0.67^{b}
	Injection	121	78.06 ± 0.13^{b}	-4.82 ± 0.01^{ab}	4.08 ± 0.00^{abc}	2.16 ± 0.18^{b}
		135	78.93 ± 0.04^{ab}	-4.74 ± 0.03^{ab}	4.48 ± 0.13^{abc}	3.13 ± 0.14^{ab}
		142	79.12 ± 0.13^{ab}	-4.62 ± 0.00^{ab}	4.71 ± 0.14^{ab}	3.41 ± 0.26^{ab}
	Tubular	121	80.58 ± 1.26^{ab}	-4.24 ± 0.92^{ab}	3.49 ± 1.00^{bc}	3.26 ± 1.41^{b}
		135	81.40 ± 1.33^{ab}	-3.86 ± 1.00^{ab}	5.12 ± 1.30^{ab}	5.08 ± 1.77^{ab}
		142	82.85 ± 1.07^{a}	-2.57 ± 1.07^{a}	7.25 ± 1.07^{a}	7.95 ± 2.49^{a}
8% Protein	Unheated		80.20 ± 0.44^{c}	-5.36 ± 0.18^{c}	2.82 ± 0.40^{e}	
	Infusion	121	81.81 ± 1.19^{abc}	-4.54 ± 0.08^{b}	3.71 ± 0.41^{d}	2.75 ± 0.62^{d}
		135	82.08 ± 1.20^{abc}	-4.52 ± 0.09^{b}	3.84 ± 0.29^{d}	3.31 ± 0.78^{cd}
		142	82.28 ± 1.20^{abc}	-4.39 ± 0.04^{b}	4.28 ± 0.28^{cd}	3.77 ± 0.74^{bc}
	Injection	121	80.14 ± 0.27^{c}	-4.51 ± 0.02^{b}	5.22 ± 0.16^{bc}	1.36 ± 0.42^{e}
		135	80.72 ± 0.08^{bc}	-4.38 ± 0.04^{b}	5.58 ± 0.10^{b}	2.03 ± 0.03^{de}
		142	80.92 ± 0.22^{bc}	-4.26 ± 0.02^{b}	5.85 ± 0.19^{b}	2.38 ± 0.43^{cde}
	Tubular	121	81.90 ± 0.80^{abc}	-4.79 ± 0.16^{b}	4.13 ± 0.34^{cd}	2.90 ± 0.37^{cd}
		135	82.57 ± 0.78^{ab}	-4.44 ± 0.38^{b}	5.53 ± 0.80^{b}	3.95 ± 0.09^{b}
		142	84.04 ± 1.40^{a}	-3.72 ± 0.57^{a}	7.11 ± 1.22^{a}	5.87 ± 0.55^{a}

temperature, while infusion did not. For tubular-heated milks the b* value was shown to increase with increasing final heat temperature, while injection-treated milks did

not. Increases in b* values can result from the occurrence of Maillard browning in a system (Morales and van Boekel, 1998). The increased b* values in tubular- and injection- treated formulations this indicates a greater level of Maillard browning compared to that in infusion- treated formulations.

Euclidean distance, ΔE, provides information on the overall colour change from the unheated formulation for each of the heat-treated protein-enriched beverages (Table 6.3). Protein concentration, heating technology and final heat temperature all significantly affected the ΔE ($p < 0.001$, for each factor in terms of three-way ANOVA; Table 6.2.). Tubular heating resulted in the greatest overall colour change, particularly at 6 and 8% protein, and all heating temperatures resulted in a visibly observable colour difference ($\Delta E > 2.3$). It should be noted that these colour changes are not throught to be of an oder of magnitude that would be undesirable from a consumer perspective.

6.4. Conclusion

Supersonic steam injection heating provides substantial retention of native whey protein, particularly heat labile β-lg, across three ESL and UHT temperatures, compared to traditional tubular and direct steam infusion heating. Physical characteristics such as viscosity, particle size and accelerated storage stability did not significantly differ between the differently heat-treated formulations. It is well established that direct heating imparts less thermal damage on a product than indirect heating; however, the more novel supersonic direct steam injection technology provides an opportunity to further reduce thermal damage of dairy beverages. The application of this technology could enable product development opportunities for

long-life ready-to-drink high-protein beverages with high levels of native whey protein.

6.5. Acknowledgements

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Chapter 7.

Overall conclusion and suggestions for future research

7.1. Overall discussion

Protein-enriched dairy beverages form part of a growing global market servicing consumer demands for nutritionally-fortified, convenient foods. Dairy processors can deliver lucrative, value-added dairy products by leveraging the widely acknowledged high nutritional quality of dairy protein. The thermal processing required to produce ready-to-drink, protein-enriched dairy beverages for ambient delivery can pose significant technical challenges during manufacture. The increased protein content, particularly whey protein, of such beverages leads to decreased thermal stability, associated issues such as protein denaturation/aggregation, increased volatile compounds, and sedimentation during storage, affect consumer acceptance of these products. This thesis examined laboratory techniques and thermal treatment technologies which had the potential to improve the quality and process efficiency of protein dairy beverage production. To achieve this, the composition and thermal processing parameters of protein-enriched dairy beverage systems were investigated for different thermal treatment technologies.

Temperature-dependent viscosity models applied to dairy protein beverages during laboratory-scale heating were used as part of a novel method of predicting thermal stability, in terms of thermally-induced viscosity changes (Chapter 2). The methodology enables rapid, quantitative evaluation of viscosity-temperature profiles, and could be used in a product development and optimisation space to ease determination of the processing stability of dairy protein beverage formulations. This work addressed recent concerns about the widespread application of the Arrhenius equation were addressed by evaluating empirical alternatives, i.e., Generalized Arrhenius and Exponential equations (Peleg *et al.*, 2012; Peleg, 2017). It was found that the WLF equation, despite its original development for amorphous polymers, is

applicable to dairy beverage systems and provides a superior fit to viscosity data during heating than the traditionally applied Arrhenius equation (Table 2.4).

The application of direct heating technologies to high-protein beverage formulations provided significantly reduced thermally-induced changes for both whey protein- and skim milk-based protein beverages (0:100 and 80:20 CN:WP ratio, respectively). The level of whey protein denaturation in heat-treated beverages was heavily impacted by heating technology selection. For 0:100 protein solutions, infusion heating resulting in the retention of significantly greater levels of native whey protein (on average 29 and 16% of native α-la and β-lg remaining, respectively) in the beverages than tubular heating (on average 3% of native α-la and β-lg remaining) (Chapter 4). Similarly for protein-enriched skim milk-based beverages (80:20 CN:WP), infusion technology (on average 70 and 24% of native α-la and β-lg remaining) and tubular heating (on average 28 and 3% of native α-la and β-lg remaining) resulted in substantially different levels of native whey protein. Even more striking was that novel SSIH-treatment produced beverages with levels of whey protein denaturation which were lower than even traditional direct steam infusion technology (on average 82 and 66% of native α-la and β-lg remaining). It should be noted that processing of whey protein-based beverages using SSIH technology was attempted but could not be completed due to fouling within the injector preventing steady state flow being achieved, which may be due to the high levels of shear present in the SSIH injector (Murphy *et al.*, 2011; Murphy *et al.*, 2013). Addition of a small proportion of casein and utilisation of the associated chaperone-effect may improve the processing of whey protein-based beverages using SSIH technology.

Reduction in the degree of whey protein denaturation is associated with a reduction in the in the level of sulphur compounds and strong 'cooked' flavours (Al-Attabi *et al.*, 2008). In Chapter 4, infusion-treated protein beverages had a volatile profile which was closer to the unheated control, than indirect-treated variants, and a significant reduction in volatile compounds associated with 'off-flavours'. The application of direct heating technology has the potential to produce long shelf-life dairy beverages with significantly reduced off-flavours and provide competitive advantage to dairy processors who choose to adopt the technology.

The degree of whey protein denaturation was shown to be influenced by the type of heat treatment technology employed (Chapters 4, 5 and 6), but also by the CN:WP ratio of the beverage formulation (Chapter 3). In Chapter 3, it was found that an increased proportion of casein reduced the degree of α-la denaturation for tubular heat-treated 3.3% (w/w) protein solutions. This trend was seen across different protein concentrations and heating technologies, where protein solutions with a CN:WP ratio of 80:20 had a higher level of native α-la post-heat treatment compared to those with a ratio of 0:100. For tubular-heated protein solutions presented in this thesis, solutions with an 80:20 CN:WP ratio had an average of 13 times more native α-la after heat treatment than 0:100 solutions (Chapters 3, 4, 5 and 6). For infusion-treated protein solutions, this trend was still evident but the difference in native α-la between 80:20 and 0:100 protein solutions was reduced with 80:20 solutions retaining 2.45 times more native α-la. This is likely due to the increased levels of native whey protein retention achieved for both 80:20 and 0:100 protein solutions through the application of infusion heating (Chapter 4 and 6).

For tubular-heated 80:20 protein solutions, the level of native α-la retained after heat treatment decreased with increasing protein concentration ($p < 0.05$). This same effect was not observed for 80:20 solutions subjected to infusion or injection heating, or for tubular- and infusion-treated 0:100 solutions. This indicates that tubular heating may not be an appropriate heating technology selection for high-protein formulations in which limiting the degree of α-la denaturation is a concern.

In summary, the studies reported in this thesis have generated new insights into the thermal processing of protein-enriched dairy beverages. The degree of whey protein denaturation was shown to be impacted by both CN:WP ratio of the beverage and heat treatment technology selected. The design and application of novel direct SSIH technology was comprehensively studied and found to provide significant benefits over direct steam infusion and indirect tubular heating technologies for skim milk-based protein beverages. The proposed viscosity modelling methodology offers rapid, quantitative assessment of thermal stability, in terms of viscosity, for protein beverage formulations in a product development space. Overall, the outcomes of this work provide a better understanding of high heat treatment processing of heat-sensitive milk proteins, in terms of formulation manipulation and thermal treatment technologies.

7.2. Recommendations for future work

Following on from the work presented in this thesis, there are a number of complimentary studies which could be undertaken.

1. Wider application of the methodology developed in Chapter 2 could provide useful insight into the impact of factors such as mineral content and composition, pH,

preheat treatment of proteins, and protein concentration on the thermal stability, particularly in terms of viscosity, of dairy protein beverages. Extended use of the methodology could provide significant insight into optimisation of such factors for dairy protein systems;

2. Direct heat treatment can often result in the dilution or concentration of the heat-treated product due to the addition and removal of steam/condensed water as part of the heat treatment process (Chapter 4, 5, and 6). The total solids content, and therefore protein content, of dairy protein beverages could be concentrated through the adjustment of the flash cooler operational parameters after heat treatment. This could result in a reduction of the heat-treament-related issues associated with the high protein content in nutritional dairy beverages;
3. When completing the experimental work for Chapter 4, it was found that the whey protein-based beverage (0:100 CN:WP) could not be processed using SSIH technology and resulted in high degrees of system instability and fouling at the de Laval nozzle inlet of the injector. A study incrementally altering the CN:WP ratio of a whey protein-based beverage formulation could be used to determine the level of casein required to limit fouling within the nozzle and allow processing of a whey protein-based beverage with SSIH technology;
4. Following on from the work completed in Chapter 4 and Chapter 6, investigations could be completed for other dairy protein beverage formulations, such as whey-protein-enriched skim milk-based beverages, across the indirect tubular, direct infusion, and direct SSIH technology. Alteration of the CN:WP ratio has been shown to result in differences in the degree of physical property change for protein beverages (Chapter 3). Heat-treatment of WPC-enriched skim milk could provide valuable insight for dairy processors;

5. Fouling has a significant economic impact on the production of dairy products due to the costs associated with reduced heat transfer efficiency, shut down and cleaning. Direct heating is reported to result in significantly less fouling than indirect heating technologies (Lewis and Deeth, 2009). However, there are few studies which quantify the differences in fouling for different heat treatment technologies. As proposed by Truong *et al.* (2017), micro-foil heat flux sensors could be used to identify the build-up of fouling deposits within the pipe.

7.3. References

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Appendix

Peer-reviewed Scientific Articles

Evaluation of Models for Temperature-Dependent Viscosity Changes in Dairy Protein Beverage Formulations During Thermal Processing

Clodagh M. Kelleher, James A O'Mahony, Alan L. Kelly, Donal J. O'Callaghan, and Noel A. McCarthy

Abstract: Rheological modeling as a function of temperature is a useful tool for describing products undergoing thermal processing. The rheological behavior of a range of dairy-based (4%, w/w) protein beverages was investigated for applicability to semi-empirical temperature-dependent viscosity equations. The viscosity at 16.8 rad/s of the beverages was measured during heating, holding, and cooling over a temperature range of 25 to 90 °C using a rheometer with starch pasting cell geometry. Five established fitting methods were applied based on the Arrhenius and Williams–Landel–Ferry (WLF) equations using nonlinear regression analysis. A two-parameter WLF (WLF_2) model, using viscosity at a reference temperature of 25 °C resulted in high R^2 values (0.974 to 0.988) and a statistically superior fit compared to the Arrhenius, Generalized Arrhenius, and exponential equations ($P < 0.001$). Deviation from the WLF_2 modeled equation was used to describe and investigate the effect formulation had on the changes in viscosity during thermal heating. This study successfully applied the WLF equation to a liquid protein system, proving that a consistent and close fit can be achieved across a range of formulations. A rapid, quantitative method for viscosity–temperature profile evaluation is presented, which can ease product development and optimization of product processing stability.

Keywords: Arrhenius, dairy protein beverages, thermal processing viscosity, Williams–Landel–Ferry

Practical Application: This study validated the use of the Williams–Landel–Ferry equation to describe the behavior of dairy beverages during thermal processing, providing a better fit to rheological data than the widely used Arrhenius-based equations. In conjunction with the WLF equation, a method was presented which reduced the complex rheological data to a single value, which can aid in the comparison of formulations for product development and optimization in both research and industry.

Introduction

The use of whey protein ingredients in beverages for specialized applications is growing rapidly. These specialized beverages include nutritional products for the elderly, meal replacement drinks, low-sugar drinks for diabetic patients, and highly functional sports foods for high-performance athletes and body-builders (Shiby, Radhakrishna, & Singh, 2013). In addition to protein, which provides amino acids for muscle recovery and repair, these beverages often contain carbohydrates as a source of energy. Formulating such heat-stable protein-carbohydrate nutritional beverages can be challenging (Chen & O'Mahony, 2016).

Heat treatment is carried out on dairy beverages with the aim of reducing the microbial population, inactivating enzymes, while minimizing chemical reactions and physical changes in the product during storage (Lewis & Deeth, 2009). During heat treatment, a number of thermally induced physical changes occur in dairy-based beverages. Whey proteins undergo conformational changes during heating, due to unfolding of their native compact globular structures (that is, protein denaturation and aggregation), which result in technical challenges that may negatively impact process efficiency and product quality (Joyce, Brodkorb, Kelly, & O'Mahony, 2017; Wijayanti, Bansal, & Deeth, 2014). These denaturation and aggregation mechanisms, at temperatures greater than 75 °C, can lead to fouling of heat-exchangers, increased turbidity, sedimentation, and viscosity of beverages with a protein concentration greater than 3.5% (w/w; Joyce et al., 2017). Fouling is a major processing issue in the dairy industry, where up to 80% of operational costs can be related to fouling, shutdown, and cleaning processes (De Jong, 2008). Attempts to reduce fouling within thermal processing include increasing product heat stability, reducing temperature and residence time, increasing flow velocities and turbulence, and monitoring pH (Feldman, 2016; Santos, Nylander, Paulsson, & Trägårdh, 2006).

Rheological characterization is used in process engineering, quality control and product development (Messaâdi et al., 2015), and changes in rheological properties, such as viscosity, have been long-established as indicators of protein denaturation, aggregation, and fouling (Wallhäußer, Hussein, & Becker, 2012). Heat stability can also be determined from viscosity measurements at high temperatures, as the onset of coagulation can be detected by rheological analysis (Huppertz, 2016). Thus, characterizing the rheological behavior of a dairy protein beverage under a defined heating cycle can provide useful insights into its behavior during thermal processing, aiding formulation design. Mathematical modeling of viscosity can be employed to reduce a large quantity of rheological data to mathematical equations that can be related to physical changes, easing the description of this rheological behavior (Saguy, 2016; Steffe, 1996).

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A number of temperature-dependent viscosity models have been cited, the most prevalent of which are forms of Arrhenius and Williams–Landel–Ferry (WLF) models. The Arrhenius equation describes the rate of a process that increases monotonically with temperature and asymptotically approaches a constant value, and is commonly used to describe the effect of temperature on kinetics of chemical and biochemical reactions. It has been used extensively to describe the temperature-dependence of viscosity in both Newtonian and non-Newtonian materials (Recondo, Elizalde, & Buera, 2006). The Arrhenius equation has been used in dairy applications, for example, to model the kinetics of whey protein denaturation (Blanpain-Avet et al., 2016; Brodkorb, Croguennec, Bouhallab, & Kehoe 2016; Jeurnink, Verheul, Stuart, & De Kruif, 1996a; Wolz & Kulozik, 2015) and microbial inactivation (Kim, Park, & Lee, 2016; Lobacz & Kowalik, 2015), although other studies have applied this equation in examining the effect of temperature on dynamic viscosity (Alcântara et al., 2012) and the effect of viscosity on the flow behavior of flavored milk drinks (Işıklı, Dönmez, Kozan, & Karababa, 2015). However, concerns have been raised about its widespread application, as the equation is often applied empirically rather than as a fundamental physical model, which can lead to issues in its application to some chemical and biological processes in food, which do not completely follow 1st- or fixed-order kinetics (Saguy, 2016). Peleg, Normand, and Corrandini (2012) suggested that the Arrhenius equation is unsuitable for food systems as the activation energy (E_a) determined is ill-defined and unverified, and the gas constant (R) holds no bearing over a food system. It has therefore been suggested that a simplified empirical model, without E_a or R terms, such as the Generalized Arrhenius and Exponential equation, may be more appropriate (Peleg, 2017).

The WLF equation has also been proposed as an alternative for describing the temperature dependence of food systems in place of the Arrhenius equation in food literature (Sapru & Labuza, 1993; Slade, Levine, & Reid, 1991; Sopade et al., 2003), as the two adjustable parameters can provide a better fit than the single Arrhenius parameter (Peleg et al., 2012). The WLF equation was used initially to relate viscosity to temperature in amorphous materials, using glass transition temperature (T_g) as a reference (Ferry, 1980; Williams, Landel, & Ferry, 1955). Although originally used to study the viscosity of polymers, the WLF equation has been applied to food systems, such as honey (Ahmed, Prabhu, Raghavan, & Ngadi, 2007; Mossel, Bhandari, D'Arcy, & Caffin, 2000; Sopade et al., 2003) and a variety of dairy applications, such as modeling nonenzymatic browning in nonfat milk and whey protein powders (Buera & Karel, 1993). It also has been used to estimate the activation energy during whey protein gelation (Katsuta & Kinsella, 1990), and to model viscoelasticity in whey protein/lactose systems (Dissanayake, Ramchandran, Piyadasa, & Vasiljevic, 2013), and crystallization (Peleg, 1992; Roos and Karel, 1992) and stickiness in amorphous lactose (Paterson, Brooks, Bronlund, & Foster, 2005; Roos & Karel, 1992). Although the application to beverage systems in this study falls outside the originally specified use for amorphous and super-cooled liquids, the model is applied in an empirical fashion, in a similar manner to applications cited for other dilute food systems (Rao, 2013).

The rheological behavior of foods, such as protein-rich beverages, is complex and influenced by numerous mechanisms and factors, but the fitting of appropriate mathematical equations could make the comparison between product composition and the effects of heat treatment easier (Steffe, 1996). The aim of this study was to investigate the application of the WLF-based equations to the viscosity–temperature profiles of dairy protein formulations, comparing then against the well-established and modified versions of the Arrhenius equation, and to investigate the application of an appropriate viscosity model to reduce rheological data to a single value as a means of accelerating formulation development of heat-treated protein-rich beverages.

Materials and Methods

Materials

Whey protein isolate (BiPro®; composition: 91.8% [w/w], protein, 0.2% [w/w], fat, 2.0% [w/w], ash and <0.2% [w/w], lactose) was supplied by Davisco Foods International (Le Sueur, MN, U.S.A.). Skim milk powder (SMP; composition: 39.9% [w/w], protein, 0.9% [w/w], fat, 46.6% [w/w], lactose, and 7.9% [w/w], ash) was supplied by Tipperary Co-operative (Tipperary Town, Co. Tipperary, Ireland). Lactose was supplied by Glanbia Ingredients Ireland Ltd. (Ballyragget, Co. Kilkenny, Ireland). Maltodextrin (moisture and ash levels of <5.0% [w/w] and <0.5% [w/w], respectively, and a dextrose equivalent value of 16) was supplied by Cargill CTS (Haubourdin, France).

Model beverage formulation

Five formulations were produced with two experimental design variables: protein source, that is, whey protein or a 60:40 blend of whey protein and casein, and carbohydrate content, that is, varying lactose and maltodextrin levels (Table 1). The total protein content of each formulation was 4% (w/w). Distilled water was heated to 42 °C to aid reconstitution of the powder ingredients, which were subsequently added and mixed using a magnetic stirrer at approximately 200 rpm for 2 hr. The pH of the formulation was adjusted to 6.8 using <500 μL of sodium hydroxide and/or hydrochloric acid solutions at 0.1 M, if required. After mixing, the samples were stirred gently overnight at 4 °C to ensure complete hydration. Experimental batches were prepared separately, in triplicate.

Rheological analysis

The rheological behavior of the formulations under shearing conditions was determined using an AR 2000ex rheometer (TA Instruments, Crawley, U.K.) with a concentric cylinder geometry. A shear rate sweep was completed for all five formulations from 0 to 200 1/s at 25 °C and the Power Law was applied to the shear rate versus shear stress measurements:

$$\log\sigma = \log K + n\log\gamma \quad (1)$$

The value of n, a dimensionless number, indicates a fluids closeness to Newtonian flow at a value of 1. The rheological behavior of all five formulations under shear conditions was determined using the Power Law, resulting in n values between 1.03 and 1.06. As a result, the formulations can be considered relatively Newtonian under various shear rate conditions at a constant temperature, therefore, validating the use of viscosity data at the single chosen angular velocity.

Apparent viscosity as a function of temperature was measured using the AR 2000ex rheometer paired with a starch pasting cell geometry, which was selected for rapid heating and cooling capabilities. Temperature was controlled by peltier heating and air–water coolant circulation, as required. A temperature sweep was performed at a constant angular velocity of 16.8 rad/s over a

Table 1–Dairy protein beverage formulation composition (g/100 g).[a]

Formulation[b]	W	WL	WCL	WLM	WCLM
Whey protein	4.00	4.00	2.40	4.00	2.40
Casein	0.00	0.00	1.60	0.00	1.60
Lactose	0.00	2.35	2.35	2.35	2.35
Maltodextrin	0.00	0.00	0.00	2.35	2.35
Total solids	4.35	6.70	7.16	9.05	9.51

[a]The composition is calculated from the amount of each ingredient added, based on known ingredient composition, given in Section 2.1.
[b]The formulations are labeled with regard to their composition: whey protein only (W), whey protein and lactose (WL), whey protein/casein and lactose (WCL), whey protein and lactose/maltodextrin (WLM), and whey protein/casein and lactose/maltodextrin (WCLM).

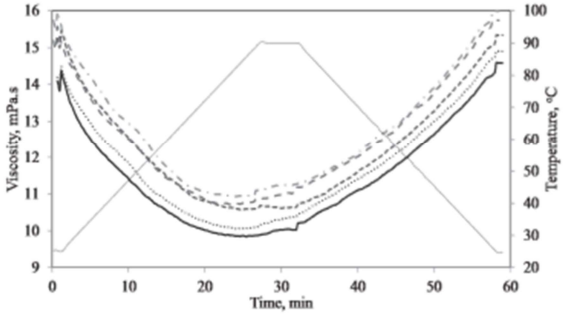

Figure 1–Viscosity profiles during heating from 25 to 90 °C, holding at 90 °C for 5 min and cooling from 90 to 25 °C (temperature profile shown by ——) for whey protein beverage formulations W (——), WL (——), WCL (----), WLM (– – –), and WCLM (– – –), illustrating the slowed decrease in viscosity and onset of increasing viscosity from approximately 70 °C during the upward temperature ramp.

Table 2–The slope of temperature segments of the upward, holding and downward temperature ramps of viscosity profiles of the beverage formulations in mPa·s/min

Formulation	Increasing temperature		Hold	Decreasing temperature	
	25 to 70 °C	70 to 90 °C	90 °C	90 to 70 °C	70 to 25 °C
W	−0.22	−0.03	0.03	0.12	0.19
WL	−0.22	−0.03	0.04	0.13	0.19
WCL	−0.24	−0.04	0.01	0.11	0.20
WLM	−0.24	−0.03	0.04	0.11	0.21
WCLM	−0.26	−0.02	0.02	0.10	0.21

heating step from 25 to 90 °C at 2.5 °C/min, a holding step at 90 °C for 5 min, and a cooling step to 25 at 2.5 °C/min. Previous work carried out by Feldman (2016) showed that the Microthermics tubular heat exchanger system operates with transitional flow at a flow rate of 3 L/min, as does the starch pasting cell when rotating at an angular velocity of 16.8 rad/s, based on Reynolds number calculations. The transitional flow behavior was validated using Reynolds number, between Re 2000 and 4100. Similar flow profiles between the two methods allow for the simulation of pilot-scale processing conditions at lab scale and are usefulness in measuring viscosity of protein formulations (Joyce et al., 2017; Murphy, Fenelon, Roos, & Hogan, 2014).

Curve fitting to temperature-dependent viscosity models

Established models were fitted to viscosity data for each upward and downward temperature sweep using the generalized reduced gradient (GRG Nonlinear) algorithm in the Solver add-in of Microsoft Excel 2010 (Lasdon, Fox, & Ratner, 1974). The model parameters were determined by least squares regression, minimizing the sum of squared residuals (SSR):

$$\mathrm{SSR} = \sum_{j=1}^{n_p} (\mu_j - \mu)^2 \quad (2)$$

where n_p is the number of data points in the temperature ramp being fitted, μ_j is the viscosity (Pa·s) measured at the jth instant of time, and μ is the corresponding model prediction of viscosity (Tibäck, Langton, Oliveira, & Ahrné, 2014).

Arrhenius-based models. The Arrhenius equation, applied to viscosity, can be expressed in its simplest form as:

$$\ln \mu\,(T) = \ln \mu_o + E_a/RT \quad (3)$$

where μ is viscosity (Pa·s), μ_o is an asymptotic viscosity as T approaches infinity, E_a is the activation energy for the reaction (kJ/mol), R is the gas constant (8.314 J/mol/K), and T is the absolute temperature (K).

A Generalized Arrhenius equation has been proposed by Peleg (2017):

$$\ln \mu(T) = \ln \mu_e + a \quad (4)$$

Table 3–Parameters determined for temperature-dependent viscosity models fitted to the viscosity data of beverage formulations W, WL, WCL, WLM, and WCLM during the (A) upward and (B) downward temperature ramps

(A)	Arrhenius		Generalized Arrhenius			Exponential		WLF$_4$			WLF$_2$	
Form.	μ_o ln(Pa·s)	E_a kJ/mol	μ_o ln(Pa·s)	T_o K	d –	A –	B –	μ_o ln(Pa·s)	T_o K	C_1 –	C_2 K	C_1 –	C_2 K
W	1.65 ± 0.16	5215 ± 277	18.3 ± 0.48	259 ± 0.25	620 ± 27.3	−4.47 ± 0.01	5.69E−03 ± 2.33E−04	15.6 ± 0.28	289 ± 5.99	4.87 ± 0.04	4.49 ± 0.93	0.61 ± 0.01	36.7 ± 3.51
WL	1.68 ± 0.34	5253 ± 547	19.0 ± 1.02	260 ± 0.50	632 ± 65.8	−4.44 ± 0.01	5.83E−03 ± 6.47E−04	15.4 ± 0.45	289 ± 4.05	4.94 ± 0.04	7.08 ± 1.05	0.70 ± 0.07	52.8 ± 4.04
WCL	1.65 ± 0.16	5427 ± 323	20.4 ± 1.01	260 ± 0.35	653 ± 38.9	−4.38 ± 0.02	6.03E−03 ± 4.28E−04	15.5 ± 0.73	289 ± 23.5	4.96 ± 0.20	9.89 ± 7.43	0.77 ± 0.20	59.3 ± 29.1
WLM	1.82 ± 0.49	5246 ± 815	20.2 ± 1.79	260 ± 0.79	631 ± 98.1	−4.09 ± 0.07	5.81E−03 ± 9.14E−04	13.5 ± 3.10	287 ± 11.5	4.87 ± 0.15	7.03 ± 4.00	0.68 ± 0.17	49.2 ± 9.63
WCLM	1.66 ± 0.40	5354 ± 706	21.3 ± 1.77	260 ± 0.63	669 ± 85.0	−4.04 ± 0.07	6.17E−03 ± 7.98E−04	13.6 ± 2.98	287 ± 11.2	4.95 ± 0.15	10.1 ± 4.52	0.82 ± 0.22	65.1 ± 21.0
(B)	Arrhenius		Generalized Arrhenius			Exponential		WLF$_4$			WLF$_2$	
Form.	μ_o ln(Pa·s)	E_a kJ/mol	μ_o ln(Pa·s)	T_o K	d –	A –	B –	μ_o ln(Pa·s)	T_o K	C_1 –	C_2 K	C_1 –	C_2 K
W	2.00 ± 0.00	4903 ± 26.1	19.5 ± 0.29	293 ± 0.18	590 ± 3.14	−4.38 ± 0.01	5.45E−03 ± 3.12E−05	20.2 ± 1.55	293 ± 5.63	5.47 ± 0.04	35.6 ± 3.25	1.22 ± 0.09	158 ± 15.5
WL	2.07 ± 0.07	4872 ± 84.7	19.8 ± 0.30	293 ± 0.17	587 ± 10.2	−4.36 ± 0.01	5.41E−03 ± 1.01E−04	19.9 ± 1.75	293 ± 30.1	5.52 ± 0.29	41.7 ± 19.3	1.36 ± 0.49	184 ± 86.4
WCL	2.01 ± 0.09	5007 ± 177	20.5 ± 0.73	294 ± 0.35	603 ± 21.3	−4.15 ± 0.17	5.56E−03 ± 2.02E−04	16.5 ± 6.78	290 ± 21.5	5.40 ± 0.20	34.2 ± 12.1	1.19 ± 0.20	146 ± 27.9
WLM	2.17 ± 0.13	4887 ± 181	20.9 ± 0.56	294 ± 0.32	588 ± 21.8	−4.04 ± 0.02	5.42E−03 ± 2.08E−04	15.6 ± 5.85	289 ± 19.8	5.23 ± 0.18	27.1 ± 9.49	1.14 ± 0.17	145 ± 26.0
WCLM	2.20 ± 0.12	4875 ± 185	21.0 ± 0.70	294 ± 0.31	587 ± 22.2	−4.03 ± 0.03	5.40E−03 ± 2.15E−04	14.9 ± 5.37	288 ± 28.1	5.17 ± 0.23	24.4 ± 11.6	1.06 ± 0.26	130 ± 43.0

where T and T_o are in K, and the constant a replacing the E_a/R term in the standard Arrhenius equation has units of temperature. Peleg (2017) argues against the use of an explicit E_a/R term in complex food systems.

Peleg applied the Saravacos exponential equation in log-transformed form:

$$\ln \mu(T) = A - BT \quad (5)$$

where A and B are constants and T is in °C (Peleg, 2017; Saravacos, 1977).

The Arrhenius, Generalized Arrhenius equations express linear behavior in ln $\mu(T)$ compared with 1/T plots, often described as Arrhenius-type behavior (Messaâdi et al., 2015). The Generalized Arrhenius and Exponential equations are presented here as alternatives to the simple Arrhenius equation, replacing the E_a and R terms with alternative constants and thereby removing the issues related to the physical meaning of a "mole" in liquid food systems (Peleg, 2017; Saguy, 2016).

WLF model. The WLF equation produced by Williams et al. (1955) was expressed as:

$$\ln \mu(T) = \ln \mu(T_s) - C_1(T - T_s)/(C_2 + T - T_s) \quad (6)$$

where C_1 (–) and C_2 (K) are constants, and T_s is a selected reference temperature (K).

In this study, the WLF equation was applied in two ways, namely a WLF 4-parameter (WLF$_4$) and a WLF 2-parameter (WLF$_2$) approach. The WLF$_2$, as recommended by Peleg (1992), uses a reference temperature (T_s) of 25 °C, where $\mu(T_s)$ is the measured viscosity at 25 °C, giving two fitting parameters, C_1 and C_2, fitted using nonlinear least squares regression. The WLF$_4$ method optimizes all four parameters in Eq. (7) using this regression technique: C_1, C_2, T_o, and μ_o.

$$\ln \mu(T) = \ln \mu_o - C_1(T - T_o)/(C_2 + T - T_o) \quad (7)$$

Parameters C_1 and C_2 are determined through linear regression in both the WLF$_4$ and WLF$_2$ applications.

Statistical analysis

R^2 values were used to compare the fits of different viscosity models. Steiger's Z-test statistic was used to determine if correlations were overlapping or different and identify statistical significance of R^2 results (Steiger, 1980). All calculations were carried out using Microsoft Excel 2010. One-way ANOVA was applied to evaluate statistical differences in determined coefficients and R^2 values for formulations using the MINITAB® 15 (Minitab Ltd., Coventry, U.K.) statistical analysis package.

Results and Discussion

Viscosity–temperature profiles

Viscosity–temperature profiles exhibited similar overall trends for all five formulations; viscosity generally decreased as temperature increased, and subsequently increased as temperature decreased (Figure 1 and Table 2). However, at the higher temperatures of the upward temperature ramp, deviation from this behavior was observed, that is, at approximately 70 °C and above, the decline in viscosity slowed and viscosity began to increase, even as temperature increased. This is likely the result of whey protein denaturation and aggregation contributing to increased viscosity of the formulations, as these mechanisms are known to occur at

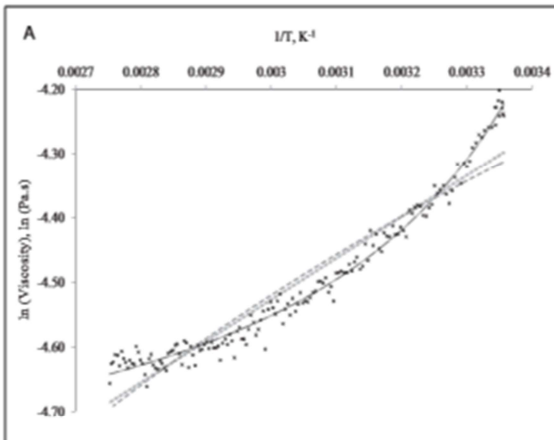

Figure 2–Viscosity data (·), Arrhenius (—), Generalized Arrhenius (---), Exponential (···), WLF_4 (-·-), and WLF_2 (—) parameter models for the (A) upward and (B) downward temperature ramps for formulation W plotted on a logarithmic scale.

Table 4–R^2 statistical analysis of temperature-dependent viscosity models fitted to the viscosity data of beverage formulations W, WL, WCL, WLM, and WCLM during the (A) upward and (B) downward temperature ramps

(A) Formulation	Arrhenius	Generalized Arrhenius	Exponential	WLF_4	WLF_2
W	0.93 ± 0.03	0.94 ± 0.02	0.91 ± 0.03	0.98 ± 0.00	0.98 ± 0.00
WL	0.94 ± 0.02	0.94 ± 0.02	0.92 ± 0.02	0.98 ± 0.01	0.98 ± 0.01
WCL	0.95 ± 0.01	0.95 ± 0.01	0.92 ± 0.01	0.98 ± 0.01	0.98 ± 0.01
WLM	0.93 ± 0.02	0.93 ± 0.02	0.91 ± 0.02	0.98 ± 0.01	0.97 ± 0.00
WCLM	0.95 ± 0.00	0.95 ± 0.00	0.92 ± 0.01	0.97 ± 0.01	0.97 ± 0.01
(B) Formulation	**Arrhenius**	**Generalized Arrhenius**	**Exponential**	**WLF_4**	**WLF_2**
W	0.99 ± 0.00	0.99 ± 0.00	0.98 ± 0.00	0.99 ± 0.00	0.99 ± 0.00
WL	0.98 ± 0.00	0.98 ± 0.00	0.98 ± 0.00	0.98 ± 0.00	0.98 ± 0.00
WCL	0.99 ± 0.00	0.99 ± 0.00	0.98 ± 0.01	0.99 ± 0.00	0.99 ± 0.00
WLM	0.98 ± 0.00	0.98 ± 0.00	0.97 ± 0.01	0.99 ± 0.00	0.99 ± 0.00
WCLM	0.98 ± 0.01	0.98 ± 0.01	0.97 ± 0.01	0.99 ± 0.00	0.99 ± 0.00

temperatures exceeding 70 °C, resulting in changes in viscosity (Chevallier et al., 2016; Joyce et al., 2017). Fitzsimons, Mulvihill, and Morris (2007), using the same commercial WPI as used in this study, found by differential scanning calorimetry analysis that, at 3.0% w/w whey protein, denaturation of β-lactoglobulin (β-lg) and α-lactalbumin (α-la) was initiated at approximately 75 °C and approximately 62 °C, respectively. Joyce et al. (2017) reported extensive protein denaturation, in a study of model infant nutritional product formulations (5.2% [w/w] protein with a whey protein: casein ratio of 60:40 formulated using the same commercial WPI and low-heat SMP) heated at 85 °C for 2 min, with a total protein denaturation level of 81.2% and β-lg denaturation

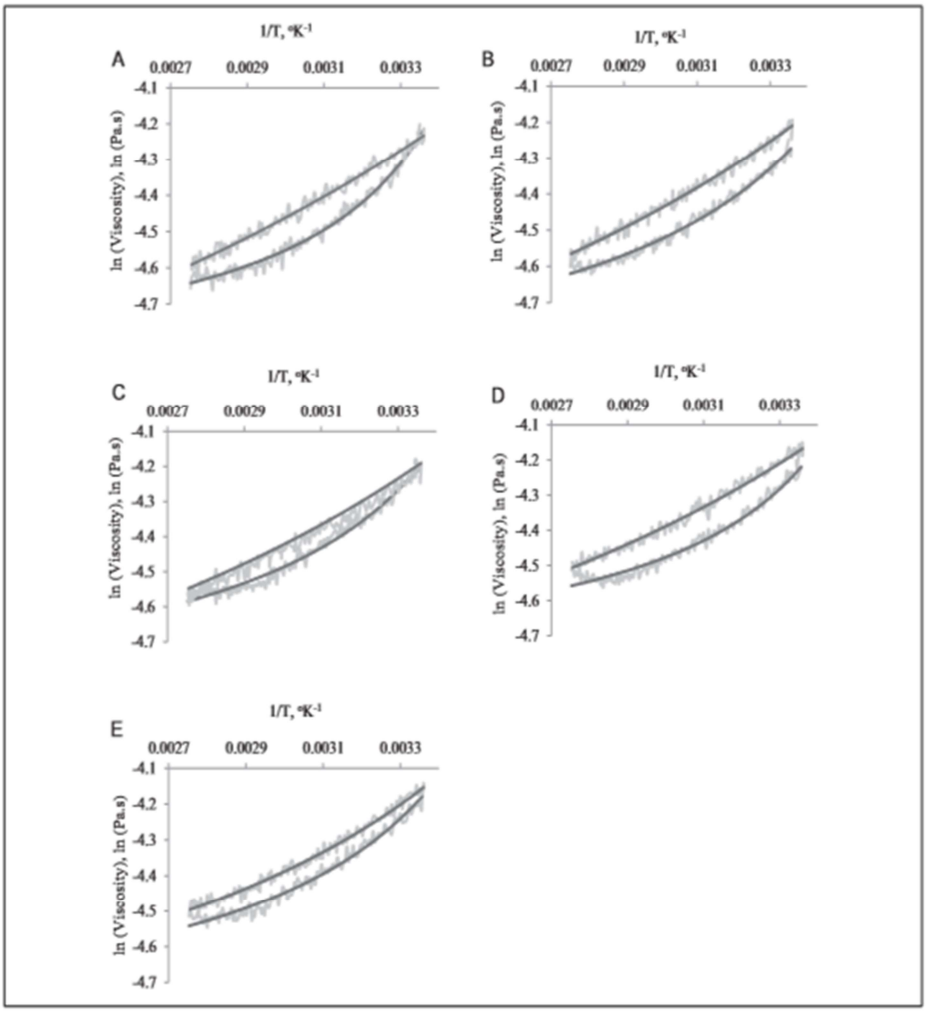

Figure 3–WLF_2 equation (—) applied to viscosity on a logarithmic scale over upward and downward temperature ramps (—) for the beverage formulations, (A) W, (B) WL, (C) WCL, (D) WLM, and (E) WCLM.

of 94.7%. In our study, the downward temperature ramp did not display the same changes in rheological behavior as the upward temperature ramp, and viscosity increased continuously as temperature decreased through the range 90 to 25 °C. In general, the viscosity of formulations increased with increasing total solids content (Table 1), as reported by others (Patocka et al., 2006).

Arrhenius-based models

The parameters determined for the Arrhenius-based equations (Arrhenius, Generalized Arrhenius, and Exponential equations) investigated did not differ to a significant degree between the beverage formulations ($P > 0.05$), with the exception of the A term of the Exponential equation (Table 3). The A values determined decreased with increasing total solids concentration of the beverage formulations, and a statistical difference between A parameters was determined for formulations containing maltodextrin (that is, WLM and WCLM) and those without ($P < 0.05$).

The downward temperature ramp was well described in all cases by the Arrhenius equation ($R^2 = 0.981$ to 0.986) and its derivatives, the Generalized Arrhenius equation ($R^2 = 0.981$ to 0.986) and the Exponential equation ($R^2 = 0.971$ to 0.979). However, a relatively poorer fit was obtained when the equations were fitted to the upward temperature ramp, with R^2 fits ranging from 0.933 to 0.947 for the Arrhenius and Generalized Arrhenius equations, and 0.908 to 0.924 for the Exponential equation (Figure 2 and Table 4). For both upward and downward temperature ramps, the Arrhenius equation provided a significantly superior fit to the Exponential equation ($P < 0.001$). The R^2 values obtained for the Arrhenius equations were greater than those for the generalized Arrhenius equation for all formulations ($P < 0.001$), with the

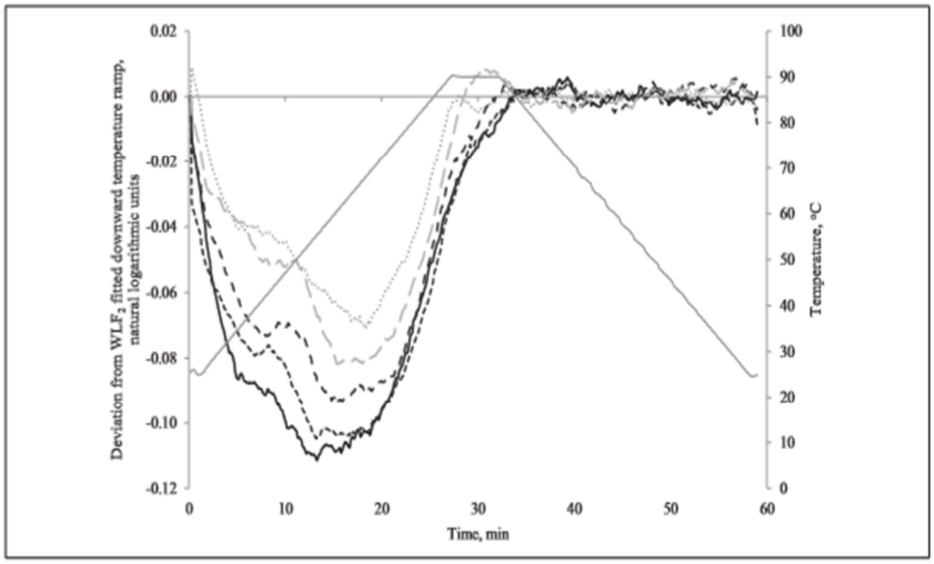

Figure 4–Deviation in viscosity from the downward temperature ramp WLF_2 modelled across the heating cycle for formulations W (—), WL (····), WCL (····), WLM (- - -), and WCLM (— —), illustrating the effect of temperature (—) on the viscosity of the formulations during the upward temperature ramp. Deviation was calculated as $(\ln[\mu_j] - \ln[\mu(T)])$, where μ_j is the viscosity at the jth instant of time, and $\mu(T)$ is the viscosity at the same temperature (T) according to WLF_2 model.

exception of *W* on the upward temperature ramp where an equal fit was obtained using both equations ($P > 0.05$).

WLF equation

Each of the parameters identified for the WLF_4 and WLF_2 equations were statistically similar across formulations ($P > 0.05$; Table 3). A linear relationship was found between C_1 and C_2 for all formulations that was characteristic of upward and downward temperature ramps; a different relationship was found for WLF_4 and WLF_2 models. It can be inferred from Equation (6) that the curvature of a plot of log viscosity versus $1/T$ is related to C_2 and that curvature becomes insignificant, that is, tends toward a straight line, when C_2 is much greater than the maximum value of $T - T_s$. The coefficient C_1 is influenced by C_2 and the overall slope of the plot. This effect can be seen in all formulation models, where lower C_2 values are reflected in greater curvature for the upward temperature ramp (Figure 3 and Table 3). The fits obtained using the WLF equations, WLF_4 and WLF_2, were far superior to those using Arrhenius-based equations in all cases on upward and downward temperature ramps ($P < 0.001$; Table 4). This was particularly evident for the upward temperature ramp, where the average R^2 values of both WLF equations was 0.978 compared to an average of 0.933 for the three Arrhenius-based equations (Figure 2). The downward temperature ramp was well described by most models, with the average R^2 value exceeding 0.983, with the exception of the Exponential equation (R^2 of 0.975); however, the application of the WLF equations provided the best fit (R^2 of 0.986).

Both the WLF_4 and WLF_2 equations provided statistically similar fits for the downward temperature ramp of all formulations ($P > 0.001$; Figure 2 and Table 4). On the upward temperature ramp, the WLF_4 equation gave a fit that was as good as, or in the case of the W and WLM formulations, better than that of the WLF_2 equation ($P < 0.001$). The WLF_2 equation allows for comparison of viscosity-temperature relationships for formulations using only two calculated parameters and maintains the relationship between T_s and μ_s, unlike the WLF_4 equation for which these values are determined by regression. As the fit provided by the WLF_4 and WLF_2 equations is similar in most cases, the more parsimonious WLF_2 equation is preferred over the other equations applied in this study. Despite its original development for amorphous polymers, the WLF equation was successfully applied empirically to liquid dairy-protein formulations, resulting in a better fit than the traditionally applied Arrhenius-based equations.

Application of the WLF_2 equation to the difference in viscosity on heating and cooling and its use in comparing formulations. From the application of the various models to the range of formulations in this study, it could be seen that the cooling ramps of all formulations exhibited an inverse viscosity–temperature relationship (Figure 3 and Table 4). The deviation of viscosity during the heating ramp from the Arrhenius-type behavior exhibited by the cooling ramp can be attributed to protein denaturation and aggregation during the heating step. Thus, the deviation of the heating temperature ramp from the WLF_2 fitted cooling temperature ramp can be taken as a relative measure of the contribution of denaturation/aggregation to viscosity for the respective formulations (Figure 4). The selected WLF_2 model was applied to the rheological data of each beverage formulation and by using integration (area under the deviation with respect to time curve in log units to base 10 × s), the extent of this influence was analyzed across the heating ramp, from 25 °C to 90 °C (that is, 2 to 27 min).

Formulations with whey protein as the only protein source (W, WL, and WLM) exhibited a greater overall increase in viscosity from heating ramp to cooling ramp (55.8, 52.8, and 45.4 log.s, respectively) than formulations containing both whey protein and casein (WCL and WCLM at 29.7 and 35.3 log.s, respectively) despite the latter formulations having higher solids

contents (Figure 4). The presence of casein and lactose in milk-based solutions has been shown to alter reaction rates and orders for whey protein denaturation, which in turn affects viscosity (Brodkorb et al., 2016). Previous studies have shown chaperone-like functions of casein, whereby the addition of casein to a whey protein system can increase heat stability and prevent irreversible aggregation of whey proteins induced by thermal processing (O'Kennedy & Mounsey, 2006). The same effect was seen by Chevalier et al. (2016), who reported that 4% protein solutions containing both whey protein and casein had increased heat stability after heat-induced aggregation of whey proteins.

The formulations containing whey protein as the sole source of protein, W and WL, had relative viscosity increases (integrated deviations of Figure 4 plots) of 55.8 and 52.8 log.s, respectively, whereas the whey protein-based formulation containing maltodextrin (WLM) showed a much lower viscosity increase, of 45.4 log.s. The addition of maltodextrin to formulation WLM resulted in less overall viscosity deviation than for W and WL. Thus, in this study, the addition of lactose had a negligible effect on viscosity, although the impact of maltodextrin was much greater. Previous studies have found that whey protein denaturation mechanisms can be retarded in the presence of sugars such as lactose (Brodkorb et al., 2016) and that increases in viscosity due to thermal processing can also be reduced by the presence of carbohydrates. Our findings are consistent with those reported by Crowley, Kelly, and O'Mahony (2014), who found that the addition of lactose to a dairy system can impair heat stability due to heat-induced acidification of the system, while maltodextrin has less impact on heat stability due to it having less reactivity during thermal processing. Similarly, Mulcahy, Park, Drake, Mulvihill, & O'Mahony (2016), found that the addition of maltodextrin can improve heat stability of WPI dispersions containing 5% (w/w) protein and 5% (w/w) maltodextrin, which had similar overall composition to the formulations investigated in this study. For formulations containing both whey protein and casein, namely WCL and WCLM, the viscosity deviations (at 29.7 and 35.3 log.s, respectively), were lower than for the whey protein-only formulations referred to above (55.8, 52.8, and 45.4 log.s); however, the addition of maltodextrin (comparing WCL and WCLM) increased, rather than reduced, the viscosity deviation (Figure 4). The approach of using a viscosity model to measure the impact of heat treatment, as outlined here, is a novel approach to quantitatively evaluating the effect of thermal processing on the viscosity of dairy formulations.

Conclusion

This study compared the novel empirical application of the WLF equation with the well-established Arrhenius equation for liquid dairy systems. The study statistically validated the use of the WLF equation for use with dairy products and showed that the WLF equation, applied empirically, can provide a better fit than the traditionally used Arrhenius equation. The Arrhenius, Generalized Arrhenius, and Exponential equations proved to be inadequate for describing the temperature-dependence of viscosity in a system where heat-induced protein denaturation and aggregation occurs. The condensing of temperature-related viscosity data using the WLF equation allows for the prediction of thermal-induced viscosity issues. This model allows processors (that is, dairy beverage and infant formula producers) to make informed assessments on the thermal stability of their products in terms of viscosity. Future work could also be extended to model the effects of minerals, pH, and protein concentration on the viscosity behavior of dairy formulations during thermal processing.

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Authors' Contributions

Clodagh M. Kelleher collected data, carried out application of mathematical models and drafted the manuscript. James A. O'Mahony designed the study, interpreted results, and drafted the manuscript. Alan L. Kelly interpreted results and drafted the manuscript. Donal J. O' Callaghan performed mathematical modeling and statistical analysis, and drafted the manuscript. Noel A. McCarthy collected data and drafted the manuscript.

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The effect of direct and indirect heat treatment on the attributes of whey protein beverages

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ABSTRACT

Thermal processing of ready-to-drink high protein beverages can have a substantial impact on the physical and sensory properties of the final product for long-life milks such as extended shelf life and ultra high temperature processed products. Direct and indirect heat treatment technologies were applied to whey protein isolate (WPI) -based beverages containing 4, 6 or 8% (w/w) protein. Lower levels of protein denaturation (66–94%) were observed using direct heating compared with indirect heating (95–99%) across protein levels and heating temperatures (121 and 135 °C final heat). Direct heat treatment resulted in significantly lower viscosity and less extensive changes to the volatile profile, compared with indirect heat treatment. Overall, the application of direct and indirect heat treatment to WPI solutions resulted in significantly different final products in terms of appearance, physical characteristics and volatile profile, with direct heating resulting in many enhanced properties compared with conventional indirect heat treatment.

1. Introduction

Nutritional beverages are a rapidly growing market segment, with sales increasing by an average of approximately 5% annually (Chen & O'Mahony, 2016; Cochrane et al., 2012). These products can be formulated to cater for a variety of consumer needs such as functional sports foods for high performance athletes and body-builders, meal replacement drinks for dietetic nutrition, and low-sugar drinks for diabetic patients (Beecher, Drake, Luck, & Foegeding, 2008; Jelen, 2009; Shiby, Radhakrishna, & Singh Bawa, 2013).

When developing protein beverages, whey proteins are commonly used as a protein source due to their excellent nutritional qualities, bland flavour, ease of digestibility and functionality in beverage systems (Rittmanic, 2006). Formerly considered a waste by-product of cheese and casein production, whey protein has become highly valued for its nutritional and functional properties (Boland, 2011; Evans & Gordon, 1980; Fitzsimons, Mulvihill, & Morris, 2007; Mulvihill & Ennis, 2003; Smithers, 2008). However, technological processes used in dairy-based beverage manufacture may impair the high nutritional value of whey proteins, whereby protein denaturation and aggregation and loss of solubility decrease protein digestibility and bioavailability (Pellegrino, 2013). As a result, selection of thermal processing technology is an important factor affecting the level of protein denaturation and nutritional value of products, in addition to reducing aggregate-related storage stability issues in long-life products, such as increases in viscosity, turbidity and sedimentation (Le et al., 2016; Villumsen et al., 2015a and b).

Typical heat treatment processes used during manufacture of whey protein beverages are in the extended shelf life (ESL) heat treatment range (120–135 °C for 2–4 s) or ultra high temperature (UHT) range (135–145 °C for 2–4 s) (Britz & Robinson, 2008; Deeth & Lewis, 2016; Rysstad & Kolstad, 2006). There are two classical modes of high temperature short time (HTST) heating, i.e., indirect and direct heating, used for the commercial sterilisation of milk and milk products (Deeth & Lewis, 2016; Roux et al., 2016).

Indirect systems, using tubular and plate heat exchangers, promote heat transfer across an interface. For direct systems, like injection and infusion, the heating medium, steam, is in direct contact with the product and subsequently removed through flash cooling (Burton, 1994; Hsu, 1970; Lewis & Heppell, 2000; Schroyer, 1997).

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The heat transfer interface of indirect heating systems reduces the heat transfer rate and localised heating at the interface can result in higher levels of protein denaturation and fouling compared with direct systems (Akkerman et al., 2016; Karayannakidis, Apostolidis, & Lee, 2014; Murphy, Tobin, Roos, & Fenelon, 2011).

In direct heating systems, almost instantaneous heating is achieved due to the mixing of the heating medium and product. This method involves a more efficient and rapid rate of heat transfer than indirect heating, as it makes use of the latent heat of evaporation as the steam condenses, resulting in reduced residence time and a lower thermal load imparted on the product (Britz & Robinson, 2008; Datta, Elliott, Perkins, & Deeth, 2002; Dickow, Nielsen, & Hammershøj, 2012b; Karayannakidis et al., 2014; Lee, Barbano, & Drake, 2017).

In a number of studies direct heat treatment technology led to a lower level of whey protein denaturation compared with indirect heating for skim milk (Akkerman et al., 2016; Lee et al., 2017; Lyster, Wyeth, Perkin, & Burton, 1971) and whey protein concentrate (Dickow, Kaufmann, Wiking, & Hammershøj, 2012a). However, direct treatments are also reported to result in a greater average particle size and sediment formation compared with indirect systems, due to the reduced area of thermal transfer surfaces in direct systems for deposition of aggregates (Burton, 1968; Datta et al., 2002; Malmgren et al., 2017). These studies imply that aggregates that would generally adhere to hot surfaces and be found in fouling material during traditional indirect processing are still present in the final product. The rapid cooling in direct heating can remove volatiles in milk such as dissolved oxygen, heat-induced sulphur volatiles and other volatiles, in addition to removing excess water, resulting in less heat-induced flavour changes (Deeth & Lewis, 2016; Lee et al., 2017). Previous studies have identified direct heating processes as the best technological option to limit thermally-induced changes in milks (Roux et al., 2016; Van Asselt, Sweere, Rollema, & de Jong, 2008).

The heat treatment technology employed in dairy beverage production can have a significant impact on the taste, physical stability, and shelf life of the product. Little has been published in relation to the heat treatment of high protein whey solutions using direct heat treatment technology (Dickow et al., 2012a) or the comparison of direct and indirect technologies. The aim of this study was to investigate the impact of direct and indirect heat treatment technology at high temperatures (70 °C/121 °C and 80 °C/135 °C with preheat and final holding times of 30 s and 2 s, respectively) on selected physicochemical characteristics of high protein ready-to-drink whey protein beverages, and to determine if either technology produced significantly enhanced product quality.

2. Materials and methods

2.1. Materials and formulation

Model whey protein beverages were formulated at protein concentrations of 4, 6 and 8% (w/w), reflective of current market product protein concentrations, using whey protein isolate (BiPro®), supplied by Davisco Foods International (Le Sueur, MN, USA), which had a composition of 91.8% protein, 0.21% fat, 2.03% ash, and <0.2% lactose. The WPI powders were reconstituted in 150 L batches using reverse-osmosis water heated to 45 °C, to aid solubilisation of the ingredients. Ingredients were inducted using a YTRON ZC powder induction unit (YTRON Process Technology GmbH, Bad Endorf, Germany), consisting of a high-shear, rotor-stator mixer connected to a recirculation pump, with a 20 min recirculation time. The dispersion was stored in a tank equipped with an impeller and stirred at a low speed overnight at 4 °C. The pH was adjusted to pH 6.8 using 0.1 M HCl or KOH, as required, before and after overnight storage.

2.2. Heat treatment

Two pilot-scale thermal processing plants were used to carry out direct and indirect heat treatment of the WPI dispersions. Direct heating was applied using a UHT steam infusion pilot plant 422463 (APV, Silkeborg, Denmark), which consists of a plate heat exchanger for preheating followed by steam infusion and flash cooling vessel, and a plate heat exchanger for final cooling (Fig. 1a). Indirect heating was applied using a MicroThermics tubular UHT pilot plant (MicroThermics, NC, USA), consisting of two tubular heat exchangers for preheating and final heating operations and two tubular heat exchangers for initial and final cooling operations (Fig. 1b). Both the direct and indirect pilot plants were used with a preheat holding time of 30 s and a final heat holding time of 2 s (Fig. 1c). Two types of heating conditions were applied to the WPI dispersions using the direct and indirect pilot plants; 70 °C preheat with 121 °C final heat, and 80 °C preheat with 135 °C final heat. These temperature combinations are commonly used for extended-shelf-life (ESL) and ultra-heat-treatment (UHT) processes, respectively (Burton, 1994; Bylund, 1995; Rysstad & Kolstad, 2006). The temperature combinations used will be referred to as ESL (70/121 °C) and UHT (80/135 °C) to ease description.

2.3. Particle size analysis and molecular weight distribution

Particle size distribution of whey protein dispersions was determined using dynamic light scattering (DLS) with a Malvern Zetasizer Nano ZS instrument (Malvern Instruments Ltd., UK). Samples were dispersed in ultra-pure water for analysis in polystyrene disposable cuvettes. A refractive index of 1.45 was used for protein samples, while 1.330 was used for the dispersant. All samples were analysed at a temperature of 25 °C.

Size-exclusion high-performance liquid chromatography (SE-HPLC) was used to monitor the formation of heat-induced aggregates by determining the molecular weight (M_W) profile of the samples as described by Buggy, McManus, Brodkorb, McCarthy, and Fenelon (2016). The HPLC system used consisted of a Waters 2695 separation module with a Waters 2487 dual-wavelength detector at 280 nm, controlled using Waters Empower® software (Waters, Milford, Massachusetts, USA) using two columns in series (TSKgel G2000SWXL and G3000SWXL, 7.8 mm ID, 30 cm length, 5 µm particle size, Tosoh Biosciences LLC, USA) with a guard column (TSKgel SWXL, 6 mm ID × 4 cm length, 7 µm particle size).

2.4. Colour analysis

To investigate potential heat-induced changes in colour due to aggregation of heat labile proteins, colour measurements were carried out before and after heat treatment. The colour of each dispersion was measured and expressed as L*, a* and b* values using a Minolta Chroma Meter CR-400 colorimeter (Minolta Ltd., Milton Keynes, UK). The L* value indicates lightness, a* values indicate redness-greenness, and b* values indicate yellowness-blueness. Samples were loaded into a disposable cuvette and placed in front of a white calibration plate (L*, a*, b*) before measurement in triplicate.

2.5. Viscosity

Viscosity can impact final product acceptability for consumers, and was measured using an ARG2 controlled-stress rheometer (TA Instruments, Crawley, UK) equipped with concentric cylinder

geometry at 25 °C. The procedure involved the samples being pre-sheared at 500 s^{-1} for 1 min to neutralise the short-term rheological history of the formulations, followed by equilibration at 0 s^{-1} for 1 min. The shear rate was then increased from 5 to 500 s^{-1} over 2 min, held at 500 s^{-1} for 1 min and then decreased from 500 to 5 s^{-1} over 2 min (Murphy, Tobin, Roos, & Fenelon, 2013).

2.6. Protein and total solids measurement

The total solids content of the dispersions was measured using a Smart System 5, Smart Trac (CEM Corporation, Matthews, NC, USA).

Determination of total protein content of samples was carried out using the Kjeldahl method of analysis (IDF, 2001), using a nitrogen to protein conversion factor of 6.38.

For soluble protein analysis, denatured and aggregated protein material was removed by adjusting the sample to the isoelectric point at pH 4.6 using a 0.1 M acetate buffer to a final protein concentration of 2.5 g L^{-1} protein, centrifuging at 20,000 × *g* for 20 min at 4 °C and filtering through 0.2 μm low-protein binding PES filters (Agilent Technologies, CA, United States). The prepared samples were analysed using high-performance liquid chromatography (HPLC) using a Waters 2695 separation module, a Waters 2487 dual wavelength absorbance detector running on Waters Empower® software (Milford, MA, USA). Reversed-phase (RP) HPLC was completed using a PolymerX 5 μm RP-1, 150 × 4.6 mm column (Phenomenex, Cheshire, UK) as described by Kehoe, Wang, Morris, and Brodkorb (2011). α-Lactalbumin, β-lactoglobulin A and β-lactoglobulin B standards (Sigma Aldrich, Ireland) were used to calibrate the method.

2.7. Volatile analysis

Volatile compounds were identified using head-space solid phase microextraction (HS-SPME) coupled with gas chromatography-mass spectrometry (GC–MS) as described by Stefanovic, Kilcawley, Rea, Fitzgerald, and McAuliffe (2017), with some modifications. The sample volume was 4 mL and all samples were run in triplicate. Samples were processed using Shimadzu GCMS solutions software using the flavour and fragrance library (FFNSC 2) in combination with in house libraries and NIST 2011 Mass Spectral Library, AMDIS (www.amdis.net) software and linear retention indices were carried out using the method of Van Den Dool and Kratz (1963). Batch processing was carried out with metaMS (Wehrens, Weingart, & Mattivi, 2014) (www.rdocumentation.org). The unheated and heat-treated dispersions were frozen immediately after thermal processing, until required for volatile analysis.

2.8. Statistical analysis

All heat treatment trials were carried out in triplicate, and the resultant data sets were analysed using the MINITAB® 15 (Minitab Ltd., Coventry, UK) statistical analysis package. The statistical significance of treatment effects on physical characteristics investigated was evaluated by means of one-way analysis of variance (ANOVA) with Tukey and Dunnetts' post hoc analysis. Three-way ANOVA was completed using the factors: protein content, heat treatment technology, and temperature of heat treatment. A paired *t*-test was carried out on particle size data to further investigate the effect of heat treatment. Principal component analysis (PCA) of protein beverage volatiles was performed using The Unscrambler X multivariate analysis programme, v10.3 (CAMO ASA, Trondheim, Norway).

3. Results

3.1. Particle size and molecular weight distribution

3.1.1. Particle size distribution

In general, the particle size (z-average) of the protein dispersions increased as a result of heat treatment (Tables 1 and 2; $p < 0.001$). This was particularly the case in directly heated dispersions, with statistically significant increases found for directly ESL and UHT treated dispersions at 4 and 6% (w/w) protein, and for directly ESL treated at 8% (w/w) protein, according to Dunnett's post hoc analysis (data not shown). A paired t-test revealed that indirect ESL heat treatments gave a higher particle size than their indirect UHT-treated counterparts at 4%, 6%, and 8% (w/w) protein concentrations ($p < 0.05$, 0.01 and 0.001, respectively), with the distinction between ESL and UHT treatments becoming stronger with increasing protein concentration. Directly heat-treated samples showed no significant difference in particle size between ESL and UHT treatments.

3.1.2. Molecular weight distribution

The M_W profiles of the aggregates formed in the soluble fraction of the beverage dispersions was determined using size-exclusion chromatography. The M_W distributions were similar for the unheated dispersions at all protein concentrations, with high proportions of low M_W proteins relative to native proteins (Fig. 2). For all heat-treated dispersions, the proportion of low M_W aggregates decreased, while the proportion of medium- and high-M_W aggregates increased with increasing thermal load and protein concentration.

For all protein concentrations, direct ESL treatment produced the lowest proportion of high M_W aggregates (≥300 kDa) compared with all other heat treatments. In general, direct UHT, indirect ESL and indirect UHT treatments resulted in statistically similar M_W profiles for the soluble phase. The difference in the proportion of total protein with a M_W greater than 300 kDa between direct and indirect UHT treatments increased with increasing protein concentration, resulting in a significantly greater proportion of high M_W aggregates in the soluble fraction following indirect UHT treatment for 8% (w/w) protein concentration compared with those which were directly treated.

The proportion of total protein material with a M_W of 8–15 kDa decreased significantly for all heat treatments except for the direct ESL treatment at 4% protein. The proportion of protein material with a M_W of 8–15 kDa were not significantly different between direct UHT, indirect ESL and indirect UHT in most cases, although the proportion could be seen to decrease as the thermal load increased, i.e., direct UHT > indirect ESL > indirect UHT.

3.2. Colour analysis

All heat treatments resulted in a significant change in L* value or lightness, from the unheated dispersion, with the exception of ESL and UHT indirectly treated 8% (w/w) dispersion (Table 3). The protein content of dispersions, heating technology and heating temperature each had a significant effect on L* ($p < 0.001$; Table 3 and Fig. 3). For 4% protein dispersions, the lightness was similar for direct and indirect UHT heat treatments, while the corresponding direct and indirect ESL-treated dispersions were statistically different from each other. Direct ESL heat treatment at 6% (w/w) protein resulted in a significantly higher L* value than all other heat treatments for 6% (w/w) protein. Indirect UHT treatment resulted in a significantly lower L* value compared with that of all other heat treatments at 6% protein. For 8% protein dispersions, the L* of both direct heat treatments was significantly greater than after indirect heat treatments. A paired *t*-test showed that dispersions treated by indirect ESL had a higher L*

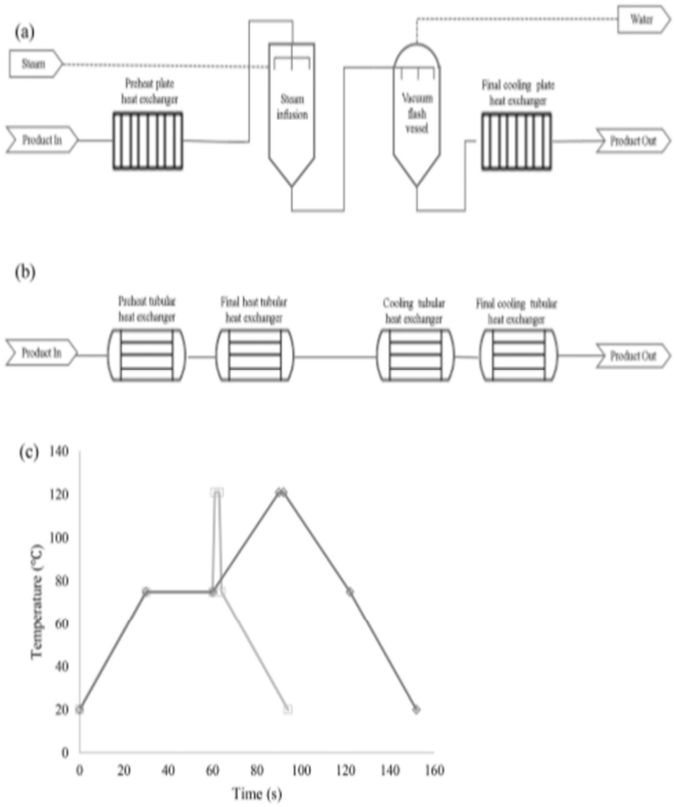

Fig. 1. Process flow diagram of (a) direct and (b) indirect heat treatment plants and (c) extended-shelf life time–temperature heating and cooling profiles of indirect (tubular heat exchanger) (—◇—) and direct (steam infusion) (—□—) heat treatment technologies.

value than their indirectly UHT-treated counterparts ($p < 0.01$). Similar to the L* value, the a* value was significantly reduced by heat treatment, implying a reduction in redness, with the exception of indirect heat treatments at 8% (w/w) protein concentration. Heat treatment significantly reduced the b* value of all protein concentrations, implying a reduction in measured yellowness (Table 3).

Table 1
Physicochemical properties of protein beverages containing 4, 6, or 8% total protein, before and after direct steam infusion and indirect tubular heat treatment.[a]

Beverage solutions	Heat treatment	pH	Total solids (%, w/w)	Total protein (%, w/w)	Soluble protein (%, w/w)	Viscosity (mPa s)	Particle diameter (nm)
4% Protein	Unheated	$6.81^{a} \pm 0.03$	$4.13^{a} \pm 0.05$	$4.10^{a} \pm 0.08$	$3.57^{a} \pm 0.10$	$3.29^{ab} \pm 0.05$	$98.2^{c} \pm 0.76$
	Direct ESL	$6.84^{a} \pm 0.04$	$3.78^{b} \pm 0.06$	$3.82^{a} \pm 0.17$	$1.72^{b} \pm 0.29$	$3.33^{b} \pm 0.04$	$278^{a} \pm 2.42$
	Direct UHT	$6.91^{a} \pm 0.03$	$3.92^{ab} \pm 0.08$	$3.96^{a} \pm 0.01$	$1.20^{c} \pm 0.11$	$3.41^{ab} \pm 0.03$	$243^{ab} \pm 38.0$
	Indirect ESL	$6.89^{a} \pm 0.02$	$4.10^{a} \pm 0.08$	$4.08^{a} \pm 0.07$	$0.75^{c} \pm 0.14$	$3.49^{ab} \pm 0.02$	$218^{b} \pm 4.60$
	Indirect UHT	$6.92^{a} \pm 0.04$	$4.06^{a} \pm 0.07$	$4.08^{a} \pm 0.06$	$0.94^{c} \pm 0.06$	$3.53^{a} \pm 0.04$	$195^{b} \pm 17.2$
6% Protein	Unheated	$6.82^{ab} \pm 0.03$	$6.37^{a} \pm 0.08$	$6.18^{ab} \pm 0.05$	$5.85^{a} \pm 0.09$	$3.37^{b} \pm 0.03$	$121^{c} \pm 4.21$
	Direct ESL	$6.77^{b} \pm 0.02$	$5.96^{a} \pm 0.08$	$5.82^{bc} \pm 0.04$	$2.19^{b} \pm 0.18$	$3.42^{b} \pm 0.02$	$192^{ab} \pm 7.77$
	Direct UHT	$6.90^{a} \pm 0.07$	$5.82^{a} \pm 0.33$	$5.61^{c} \pm 0.04$	$1.36^{c} \pm 0.14$	$3.50^{b} \pm 0.07$	$168^{b} \pm 10.9$
	Indirect ESL	$6.85^{ab} \pm 0.02$	$6.29^{a} \pm 0.10$	$6.20^{a} \pm 0.13$	$0.75^{d} \pm 0.12$	$3.91^{a} \pm 0.02$	$216^{a} \pm 0.86$
	Indirect UHT	$6.87^{ab} \pm 0.02$	$6.25^{a} \pm 0.07$	$6.22^{a} \pm 0.14$	0.96 ± 0.08^{d}	$3.69^{ab} \pm 0.02$	$136^{c} \pm 12.5$
8% Protein	Unheated	$6.81^{a} \pm 0.04$	$8.44^{a} \pm 0.06$	$8.22^{a} \pm 0.07$	$7.71^{a} \pm 0.11$	$3.42^{d} \pm 0.04$	$97.4^{ab} \pm 1.48$
	Direct ESL	$6.81^{a} \pm 0.06$	$7.83^{c} \pm 0.16$	$7.56^{b} \pm 0.19$	$3.59^{b} \pm 1.22$	$4.10^{cd} \pm 0.06$	$244^{a} \pm 11.6$
	Direct UHT	$6.82^{a} \pm 0.07$	$8.02^{bc} \pm 0.12$	$7.86^{ab} \pm 0.08$	$1.30^{a} \pm 0.09$	$4.18^{bc} \pm 0.07$	$187^{ab} \pm 83.7$
	Indirect ESL	$6.83^{a} \pm 0.05$	$8.28^{ab} \pm 0.03$	$8.13^{a} \pm 0.03$	$0.67^{c} \pm 0.02$	$9.02^{a} \pm 0.05$	$211^{ab} \pm 4.57$
	Indirect UHT	$6.86^{a} \pm 0.01$	$8.39^{a} \pm 0.03$	$8.12^{a} \pm 0.06$	$1.00^{c} \pm 0.06$	$4.61^{a} \pm 0.01$	$114^{b} \pm 1.67$

[a] For each beverage solution (protein concentration), mean values with a common superscript letter in the same column are not significantly different ($p > 0.05$). ESL refers to a 70 °C preheat temperature and 121 °C final heat temperature. UHT refers to a 80 °C preheat temperature and 135 °C final heat temperature.

Table 2
Statistical significance of the effects of target protein level, heating technology, severity of heat treatment and interactions of these factors on the physicochemical characteristics of heat treated whey protein solutions, assessed by three-way ANOVA.[a]

Characteristic		Protein level	Technology	Heat treatment	Protein level* technology	Technology* heat treatment	Protein level* heat treatment
pH		**	NS	**	NS	NS	NS
Total solids content		***	***	NS	NS	NS	NS
Total protein content		***	***	NS	**	NS	NS
Total soluble protein content		*	***	**	**	***	NS
Native protein	α-la	NS	***	***	NS	***	NS
	β-lg A	*	***	***	NS	***	NS
	β-lg B	NS	***	***	NS	*	NS
Colour coordinates	L*	***	***	***	***	*	***
	a*	***	***	***	***	*	*
	b*	*	***	NS	***	NS	*
Colour difference, ΔE		***	***	***	***	***	***
Viscosity		***	***	***	***	***	***
Particle size		***	***	***	NS	NS	NS
Molecular weight distribution	≥300 kDa	***	***	***	**	***	NS
	80–300 kDa	***	NS	NS	***	NS	NS
	30–80 kDa	***	NS	*	**	NS	NS
	15–30 kDa	***	***	***	NS	***	NS
	8–15 kDa	***	***	***	NS	***	NS

[a] Protein level refers to the target protein content to which the solutions are formulated; *** indicates $p < 0.001$,** indicates $p < 0.01$,* indicates $p < 0.05$, NS indicates no significant effect.

These changes in colour identified are visually observable and may have an impact on consumer perception.

3.3. Viscosity

Protein concentration, choice of heating technology and severity of heat treatment all had a significant effect on the viscosity of protein dispersions as determined by three-way ANOVA ($p < 0.001$; Table 2). The extent of increase in viscosity upon heating increased with increasing protein concentration of the dispersions, where the 8% (w/w) protein dispersions were the most affected by heat treatment (Table 1). Overall, direct heat treatment resulted in a lower final viscosity than indirect heat treatment, although this difference was not statistically significant in some cases below 8% protein level (Table 1).

While 4% (w/w) protein dispersions showed no significant viscosity increase on heating, the viscosity of indirectly-treated 6% (w/w) protein dispersions increased significantly with ESL treatment. At 8% (w/w) protein, heat-treated dispersions showed a significant increase in viscosity during heat treatment, with direct ESL and UHT treatments resulting in similar viscosities, which were lower than that achieved by indirect heating. Similar to the trends for 6% (w/w) protein dispersions, indirect ESL treatment of 8% (w/w) protein dispersions resulted in a significantly higher viscosity (9.02 mPa s) compared with indirect UHT treatment (4.61 mPa s), despite the higher final heating temperature in the latter. For indirect heating, there was a statistically significant interaction determined between the heating technology and heat treatment temperature ($p < 0.001$).

3.4. Protein content, profile and level of soluble protein

3.4.1. Total solids and protein content of WPI dispersions

Direct heating was associated with significantly decreased total solids contents of dispersions, with reductions of 4.95–8.58%, and the effect was particularly significant at 8% protein level (Table 1), while the total solids content was unaffected by indirect heat treatment for all protein concentrations. Three-way ANOVA analysis confirmed that heating technology had a significant effect in reducing the total solids level ($p < 0.001$), while the severity of heat treatment (i.e., ESL or UHT) did not affect total solids content (Table 2).

The total protein content of unheated and heated dispersions followed similar trends to that of total solids due to the high protein content of the WPI powder used in dispersions (Tables 1 and 2).

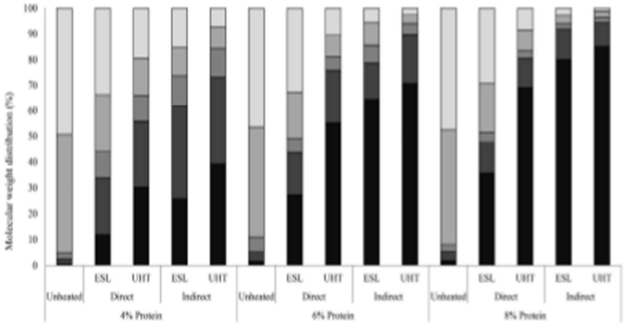

Fig. 2. Molecular weight distribution of the soluble fraction of unheated and heat-treated whey protein dispersions with molecular weights of 8–15 kDa (□), 15–30 kDa (▨), 30–80 kDa (▨), 80–300 kDa (▨), >300 kDa (■).

Table 3
Whey protein beverage colour, expressed as L*, a*, b* values for protein beverages containing 4%, 6%, or 8% total protein, before and after direct steam infusion and indirect tubular heat treatment.[a]

Solutions	Heat treatment	L*	a*	b*
4% Protein	Unheated	$39.3^{c} \pm 1.21$	$-0.65^{a} \pm 0.09$	$2.38^{a} \pm 0.35$
	Direct ESL	$64.2^{b} \pm 1.35$	$-1.46^{b} \pm 0.29$	$-5.14^{b} \pm 0.85$
	Direct UHT	$66.3^{ab} \pm 1.92$	$-1.85^{b} \pm 0.12$	$-5.27^{b} \pm 0.45$
	Indirect ESL	$68.8^{a} \pm 0.92$	$-2.30^{c} \pm 0.01$	$-6.60^{b} \pm 0.23$
	Indirect UHT	$66.5^{ab} \pm 0.80$	$-2.34^{c} \pm 0.02$	$-8.33^{c} \pm 0.47$
6% Protein	Unheated	$32.6^{d} \pm 0.82$	$-0.13^{a} \pm 0.03$	$0.76^{a} \pm 0.42$
	Direct ESL	$67.8^{a} \pm 1.30$	$-1.82^{cd} \pm 0.18$	$-5.15^{b} \pm 1.09$
	Direct UHT	$63.7^{b} \pm 2.02$	$-1.47^{c} \pm 0.23$	$-4.27^{b} \pm 0.70$
	Indirect ESL	$60.2^{b} \pm 0.77$	$-2.02^{d} \pm 0.02$	$-8.45^{c} \pm 0.21$
	Indirect UHT	$46.7^{c} \pm 0.22$	$-0.73^{b} \pm 0.04$	$-10.9^{d} \pm 0.09$
8% Protein	Unheated	$36.6^{b} \pm 0.41$	$-0.23^{a} \pm 0.07$	$2.81^{a} \pm 0.24$
	Direct ESL	$60.2^{a} \pm 1.86$	$-1.79^{b} \pm 0.11$	$-6.83^{c} \pm 0.74$
	Direct UHT	$63.6^{a} \pm 3.85$	$-1.69^{b} \pm 0.45$	$-3.09^{b} \pm 1.57$
	Indirect ESL	$41.5^{b} \pm 0.71$	$-0.32^{a} \pm 0.19$	$-7.21^{c} \pm 0.49$
	Indirect UHT	$38.1^{b} \pm 0.37$	$0.35^{a} \pm 0.08$	$-6.20^{c} \pm 0.26$

[a] For each beverage solution (protein concentration), mean values with a common superscript letter in the same column are not significantly different ($p > 0.05$). ESL refers to a 70 °C preheat temperature and 121 °C final heat temperature; UHT refers to a 80 °C preheat temperature and 135 °C final heat temperature.

While reductions in total protein content were observed for all directly heated dispersions, this reduction was only statistically significant for dispersions containing 6 or 8% (w/w) total protein. The reduction in total solids and total protein observed in directly heat-treated dispersions (i.e., steam injection and infusion) is likely the result of dilution, with condensed steam not being completely removed by flash cooling during direct processing. Product dilution, or concentration, during direct heating is common, and has been reported in numerous studies (Dickow et al., 2012a; Dumpler, Wohlschläger, & Kulozik, 2017; Lewis & Heppell, 2000; Murphy et al., 2013; Murphy et al., 2011). Net dilution or concentration within the system can be reduced by maintaining equal temperatures at preheat and flash cooling stages, and implementing finer instrument control.

3.4.2. Soluble protein

RP-HPLC showed that direct and indirect heat treatment resulted in significant levels of whey protein denaturation compared with the unheated dispersions (Fig. 4). Three-way ANOVA analysis of RP-HPLC data revealed that all protein fractions investigated were significantly affected by heating technology ($p < 0.001$) and the temperature of heat treatment ($p < 0.001$). Direct heating resulted in

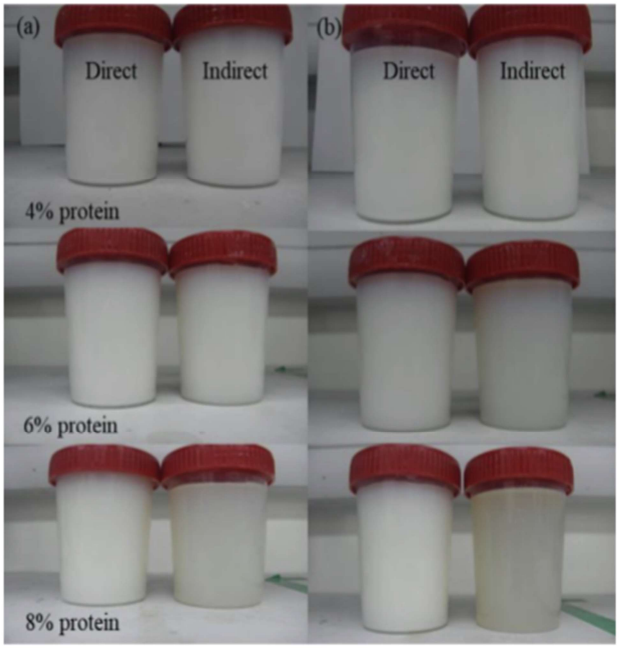

Fig. 3. Images of whey protein dispersions at 4, 6 and 8% (w/w) protein after direct and indirect with (a) ESL (70 °C preheat and 121 °C) and (b) UHT (80 °C preheat and 135 °C) heat-treated formulations.

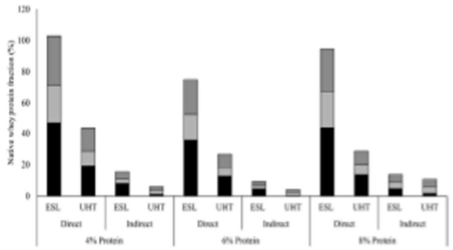

Fig. 4. Levels of native whey protein in the pH 4.6-soluble fraction measured by RP-HPLC; α-lactalbumin (■), β-lactogloblin B (□), and β-lactoglobulin A (▥) expressed as a percentage of total native whey protein for whey protein beverage dispersions at 4%, 6%, and 8% (w/w) total protein.

lower levels of protein denaturation (i.e., more native protein) for direct ESL thermal treatment in particular. Direct ESL heat treatments resulted in the retention of significantly higher levels of native α-lactalbumin (α-la) compared with indirect heating, for all dispersions tested ($p < 0.05$). The lowest level of native α-la was obtained using indirect UHT treatment, to a significant degree for the 4 and 6% (w/w) protein dispersions ($p < 0.05$). Although directly UHT-treated dispersions had a higher level of native α-la after heat treatment than indirect ESL treatment, the difference was not statistically significant in most cases (Table 1). For both the β-lactoglobulin A (β-lg A) and B (β-lg B), direct ESL treatment resulted in the lowest levels of denaturation, with the exception of the level of β-lg A in the 6% protein dispersion which, while lower, was not statistically different from that of the other heat treatments.

3.5. Volatile analysis

A range of 62 volatile aromatic organic compounds were identified in the beverage dispersions, including ketones, aldehydes, alcohols, esters, furans, sulphur- and benzene-containing compounds (results not shown). Differences between directly and indirectly treated dispersions were identified for many compounds. Indirect treatment had increased levels of aldehyde compounds ($p < 0.05$), such as pentanal, hexanal, heptanal, octanal and 2-methylpropanal, which is known to promote the 'stale' flavour in high-temperature-treated milks (Zabbia, Buys, & De Kock, 2012). A significant increase in the levels of dimethyl trisulphide and other sulphur compounds was found for indirectly heat-treated dispersions ($p < 0.05$). Such sulphur compounds are related to strong 'cooked' flavours in high temperature treated milks as a result of β-lactoglobulin denaturation (Al-Attabi, D'arcy, & Deeth, 2008). The generation of furan compounds was also noted, although the increased levels of 2-pentylfuran and 2-butylfuran with indirect heating were not significantly higher than those following direct heating.

The PCA plot shows that the volatiles profile of heat treated dispersions can be discriminated on the basis of the heating technology and severity of thermal treatment applied, particularly for indirect heat treatment (Fig. 5). The volatile profile of directly-heated dispersions related more closely to unheated dispersions than to those which were indirectly-heated. Although some differences between unheated and direct ESL dispersions could be observed, particularly for the 8% (w/w) protein dispersion, as protein concentration increased, a strong PCA grouping was not obtained with regards to ESL heat treatment applied with direct heating technology. More distinctive grouping was observed for the direct UHT treated dispersions. However, indirect heat treatment of dispersions resulted in clear differences between the unheated, ESL and UHT dispersions, which increased as the heating temperature increased. The PCA plot also showed differences based on protein content, which may have been due to a higher level of *d*-limonene found in 4% (w/w) protein dispersions than in higher protein content dispersions, although the difference levels were not statistically significant. *d*-Limonene is a terpene derived from animal feed and commonly found in milk; levels will vary dependent upon diet and metabolism in the rumen (Hansen & Heinis, 1992).

4. Discussion

The application of direct and indirect heating technologies resulted in significant differences in the physical characteristics of

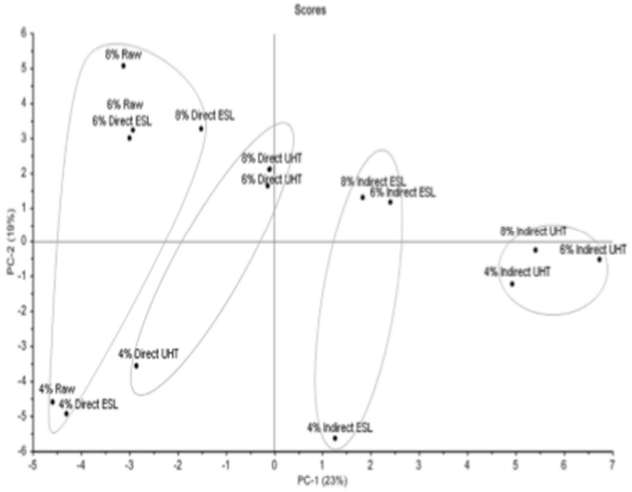

Fig. 5. Principal component analysis plot of the volatile profiles of unheated, directly and indirectly heated whey protein dispersions with 4%, 6%, or 8% total protein.

the high protein dispersions. These differences have the potential to impact consumer perception and acceptability, as they relate to protein appearance and volatile profile of the final product.

A significantly higher level of soluble protein was recorded following direct heat treatment compared with indirect heat treatment. This reduced level of protein denaturation can be attributed to the lower overall thermal load imparted due to rapid heating and cooling (Fig. 1c) (Burton, 1994; Lewis & Heppell, 2000; Murphy et al., 2013). Pellegrino, Masotti, Cattaneo, Hogenboom, and de Noni (2013) reported that the retention of a higher level of native whey proteins preserves the nutritional quality and digestibility of proteins in dispersions which may be of interest to health-conscious consumers of high protein beverages. Direct ESL treatment resulted in less protein denaturation for all dispersions, and the level of protein denaturation increased (albeit not to a significant degree in all cases) as the thermal load increased, i.e., direct ESL < direct UHT < indirect ESL < indirect UHT. These ranges are consistent with those reported in previous studies (Burton, 1994; Elliott, Dhakal, Datta, & Deeth, 2003; Lewis & Heppell, 2000).

The appearance of directly and indirectly treated dispersions was noticeably different. While directly-treated dispersions were equally opaque at each of the protein concentrations, indirectly-treated dispersions were seen to have reduced opacity as the protein concentration increased, as measured by a reduction in L* value (Fig. 3; Table 3). The significant changes in L* were consistent with the same general trends in particle size. For indirectly-treated dispersions, ESL-treated dispersions had a greater particle size and L* value than their UHT-treated counterparts, as predicted by Rayleigh's Law, which relates particle size to colour (Chung, Degner, & McClements, 2014; Desobry-Banon, Richard, & Hardy, 1994; McClements, 2002). This increased level of whiteness in whey protein dispersions obtained from direct heating systems may impact on customer perception.

Some directly-treated dispersions were found to have a larger particle size compared with indirectly-treated dispersions, despite having a lower degree of whey protein denaturation. These findings may seem counterintuitive; however, this is in agreement with the findings of previous studies (Burton, 1968; Datta et al., 2002; Malmgren et al., 2017) that proposed that the presence of some larger aggregates was related to reduced levels of deposition and fouling in direct heating systems. As the larger aggregates are not retained on heat transfer surfaces within the heating system during direct steam infusion, they remain in the product stream, contributing to increased whiteness and particle size. The difference in particle size may also be related to differences in denaturation and aggregation mechanisms due to the thermal profiles of the direct and indirect systems (Fig. 1c). Denaturation and aggregation occur in two distinct stages; the first consists of the unfolding of β-lg, and the second involves the association of these unfolded molecules to form aggregates (Joyce, Brodkorb, Kelly, & O'Mahony, 2017; Mulvihill & Donovan, 1987). Anema and McKenna (1996) found that aggregation of unfolded proteins was the rate-determining step during high-temperature processing of directly heat-treated reconstituted whole milk. The different thermal profile of the two thermal processing technologies could lead to the formation of different types of aggregates after denaturation as a result of these mechanisms.

As the average particle size of indirectly treated dispersions decreased, the viscosity of the dispersions increased, due to an increase in particle–particle interactions between a larger number of smaller particles (Table 1). Indirect ESL treatment resulted in a large increase in viscosity, from 3.42 to 9.02 mPa s, compared with both direct heat treatments and to the indirect UHT treatment, despite the higher final heating temperature. This may be due to the effect of preheating temperature, which has been shown to impact the heat stability of protein dispersions, stabilising against heat-induced physical changes during high temperature processing (Drapala, Auty, Mulvihill, & O'Mahony, 2016; Dumpler & Kulozik, 2016; Srichantra, Newstead, McCarthy, & Paterson, 2006). In this study, no such effect was seen when direct heat treatment was applied, suggesting that preheat treatment may have a less significant effect during direct heating compared with indirect.

Jansson et al. (2014) reported that the severity of heat treatments related to the development of off-flavours in milk. The results of the present study are consistent with this, as direct heat treatment, with its lower thermal load, produced a volatile profile which was closer to that of the unheated dispersion than its indirect counterpart. In addition to the reduced severity of heating during direct heat treatment, studies have shown that the rapid vacuum flash cooling step in this process can also aid in the removal of volatiles, improving the flavour of heat-treated dispersions (Deeth & Lewis, 2016; Lee et al., 2017).

5. Conclusion

The application of direct or indirect heating technology had a significant impact on the end-product functionality, appearance and sensory properties of whey protein dispersions. Direct heating resulted in many favourable product properties and significantly less thermal damage across all protein concentrations compared with indirect heating. This direct heating technology enabled the retention of higher levels of native whey protein, as determined by RP- and SE-HPLC, lower viscosity and minimal change in volatile profile. However, the products produced were more opaque than indirectly heat-treated dispersions, particularly at higher protein concentrations. Direct heat treatment can be used to process challenging whey protein beverages with a high-protein content, achieving final product properties that are unattainable with traditional indirect heat treatment methods. The application of this technology to the growing high-protein beverage market would result in products with improved nutritional value and flavour.

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